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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/435, 16/00, C07H 21/04, C12Q 1/68, G01N 33/53	A1	(11) International Publication Number: WO 96/36642 (43) International Publication Date: 21 November 1996 (21.11.96)
(21) International Application Number: PCT/US96/01078 (22) International Filing Date: 26 January 1996 (26.01.96) (30) Priority Data: 08/446,083 19 May 1995 (19.05.95) US 08/530,950 19 September 1995 (19.09.95) US (71)(72) Applicants and Inventors: DAVIS, Roger, J. [GB/US]; 53 Hickory Drive, Princeton, MA 01541 (US). GUPTA, Shashi [IN/US]; 807 Franklin Street, Worcester, MA 01604 (US). RAINGEAUD, Joel [FR/FR]; Sainte-Marie, F-85390 Bazoges-en-Pareds (FR). DERJARD, Benoit [FR/FR]; 36, rue de l'Aiguillette, Bâtiment C1, F-13012 Marseille (FR). (74) Agent: FASSE, J., Peter, Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110 (US).	(81) Designated States: AU, CA, JP, KR, MX, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>	
(54) Title: CYTOKINE-, STRESS-, AND ONCOPROTEIN-ACTIVATED HUMAN PROTEIN KINASE KINASES		
(57) Abstract <p>Disclosed are human mitogen-activated (MAP) kinase kinase isoforms (MKKs). MKKs mediate unique signal transduction pathways that activate human MAP kinases p38 and JNK, which result in activation of other factors, including activating transcription factor-2 (ATF2) and c-Jun. The pathways are activated by a number of factors, including cytokines and environmental stress. Methods are provided for identifying reagents that modulate MKK function or activity and for the use of such reagents in the treatment of MKK-mediated disorders.</p>		

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CYTOKINE-, STRESS-, AND ONCOPROTEIN-ACTIVATEDHUMAN PROTEIN KINASE KINASESBackground of the Invention

5 This invention relates to protein kinases.

Mitogen-activated protein (MAP) kinases are important mediators of signal transduction from the cell surface to the nucleus. Multiple MAP kinases have been described in yeast including SMK1, HOG1, NPK1, FUS3, and
10 KSS1. In mammals, the MAP kinases identified are extracellular signal-regulated MAP kinase (ERK), c-Jun amino-terminal kinase (JNK), and p38 kinase (Davis (1994) Trends Biochem. Sci. 19:470). These MAP kinase isoforms are activated by dual phosphorylation on threonine and
15 tyrosine.

Activating Transcription Factor-2 (ATF2), ATF α , and cAMP Response Element Binding Protein (CRE-BP α) are related transcription factors that bind to similar sequences located in the promoters of many genes (Ziff
20 (1990) Trends in Genet. 6:69). The binding of these transcription factors leads to increased transcriptional activity. ATF2 binds to several viral proteins, including the oncoprotein E1a (Liu and Green (1994) Nature 368:520), the hepatitis B virus X protein (Maguire
25 et al. (1991) Science 252:842), and the human T cell leukemia virus 1 tax protein (Wagner and Green (1993) Science 262:395). ATF2 also interacts with the tumor suppressor gene product Rb (Kim et al. (1992) Nature 358:331), the high mobility group protein HMG(I)Y (Du et
30 al. (1993) Cell 74:887), and the transcription factors nuclear NF- κ B (Du et al. (1993) Cell 74:887) and c-Jun (Benbrook and Jones (1990) Oncogene 5:295).

Summary of the Invention

We have identified and isolated a new group of
35 human mitogen-activated protein kinase kinases (MKKs).

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The MKK isoforms described herein, MKK3, MKK6, and MKK4 (including MKK4- α , - β , and - γ), have serine, threonine, and tyrosine kinase activity, and specifically phosphorylate the human MAP kinase p38 at Thr¹⁸⁰ and Tyr¹⁸². The MKK4 isoforms also phosphorylate the human MAP kinases JNK (including JNK1 and JNK2) at Thr¹⁸³ and Tyr¹⁸⁵.

Accordingly, the invention features a substantially pure human MKK polypeptide having serine, threonine, and tyrosine kinase activity that specifically phosphorylates human p38 MAP kinase. MKK3 has the amino acid sequence of SEQ ID NO:2. The invention further includes MKK6 having the amino acid sequence of SEQ ID NO:4 and having serine, threonine, and tyrosine kinase activity that specifically phosphorylates human p38 MAP kinase.

The invention further features a substantially pure human MKK polypeptide having serine, threonine, and tyrosine kinase activity that specifically phosphorylates human p38 MAP kinase and JNK. MKK4 isoform MKK4- α has the amino acid sequence of SEQ ID NO:6. MKK4 isoform MKK4- β has the amino acid sequence of SEQ ID NO:8. MKK4 isoform MKK4- γ has the amino acid sequence of SEQ ID NO:10.

As used herein, the term "mitogen-activating protein kinase kinase" or "MKK" means a protein kinase which possesses the characteristic activity of phosphorylating and activating a human mitogen-activating protein kinase. Examples of MKKs include MKK3 and MKK6, which specifically phosphorylate and activate p38 MAP kinase at Thr¹⁸⁰ and Tyr¹⁸², and MKK4 isoforms which specifically phosphorylate and activate p38 MAP kinase at Thr¹⁸⁰ and Tyr¹⁸², and JNK at Thr¹⁸³ and Tyr¹⁸⁵.

The invention includes the specific p38 MKKs disclosed, as well as closely related MKKs which are

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identified and isolated by the use of probes or antibodies prepared from the polynucleotide and amino acid sequences disclosed for the MKKs of the invention. This can be done using standard techniques, e.g., by
5 screening a genomic, cDNA, or combinatorial chemical library with a probe having all or a part of the nucleic acid sequences of the disclosed MKKs. The invention further includes synthetic polynucleotides having all or part of the amino acid sequence of the MKKs herein
10 described.

The term "polypeptide" means any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation), and includes natural proteins as well as synthetic or
15 recombinant polypeptides and peptides.

The term "substantially pure," when referring to a polypeptide, means a polypeptide that is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated.
20 A substantially pure human MKK polypeptide is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, human MKK polypeptide. A substantially pure human MKK can be obtained, for example, by extraction from a natural source; by
25 expression of a recombinant nucleic acid encoding a human MKK polypeptide, or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

30 In one aspect, the invention features isolated and purified polynucleotides which encode the MKKs of the invention. In one embodiment, the polynucleotide is the nucleotide sequence of SEQ ID NO:1. In other embodiments, the polynucleotide is the nucleotide

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sequence of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, respectively.

As used herein, "polynucleotide" refers to a nucleic acid sequence of deoxyribonucleotides or ribonucleotides in the form of a separate fragment or a component of a larger construct. DNA encoding portions or all of the polypeptides of the invention can be assembled from cDNA fragments or from oligonucleotides that provide a synthetic gene which can be expressed in a recombinant transcriptional unit. Polynucleotide sequences of the invention include DNA, RNA, and cDNA sequences, and can be derived from natural sources or synthetic sequences synthesized by methods known to the art.

As used herein, an "isolated" polynucleotide is a polynucleotide that is not immediately contiguous (i.e., covalently linked) with either of the coding sequences with which it is immediately contiguous (i.e., one at the 5' end and one at the 3' end) in the naturally-occurring genome of the organism from which the polynucleotide is derived. The term therefore includes, for example, a recombinant polynucleotide which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequences.

The isolated and purified polynucleotide sequences of the invention also include polynucleotide sequences that hybridize under stringent conditions to the polynucleotide sequences specified herein. The term "stringent conditions" means hybridization conditions that guarantee specificity between hybridizing polynucleotide sequences, such as those described herein,

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or more stringent conditions. One skilled in the art can select posthybridization washing conditions, including temperature and salt concentrations, which reduce the number of nonspecific hybridizations such that only
5 highly complementary sequences are identified (Sambrook et al. (1989) in Molecular Cloning, 2d ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

The isolated and purified polynucleotide sequences of the invention also include sequences complementary to
10 the polynucleotide encoding MKK (antisense sequences). Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub (1990) *Scientific American* 262:40). The invention includes all antisense polynucleotides
15 capable of inhibiting production of MKK polypeptides. In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and
20 introduced into a target MKK-producing cell. The use of antisense methods to inhibit the translation of genes is known in the art, and is described, e.g., in Marcus-Sakura *Anal. Biochem.*, 172:289 (1988).

In addition, ribozyme nucleotide sequences for MKK
25 are included in the invention. Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences encoding these RNAs, molecules can
30 be engineered to recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech (1988) *J. Amer. Med. Assn.* 260:3030). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

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There are two basic types of ribozymes namely, tetrahymena-type (Hasselhoff (1988) Nature 334:585) and "hammerhead"-type. Tetrahymena-type ribozymes recognize sequences which are four bases in length, while

5 "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to tetrahymena-type

10 ribozymes for inactivating a specific mRNA species, and 18-base recognition sequences are preferable to shorter recognition sequences.

The MKK polypeptides can also be used to produce antibodies that are immunoreactive or bind epitopes of

15 the MKK polypeptides. Accordingly, one aspect of the invention features antibodies to the MKK polypeptides of the invention. The antibodies of the invention include polyclonal antibodies which consist of pooled monoclonal antibodies with different epitopic specificities, as well

20 as distinct monoclonal antibody preparations. Monoclonal antibodies are made from antigen-containing fragments of the MKK polypeptide by methods known in the art (See, for example, Kohler et al. (1975) Nature 256:495).

The term "antibody" as used herein includes intact

25 molecules as well as fragments thereof, such as Fa, F(ab')₂, and Fv, which are capable of binding the epitopic determinant. Antibodies that bind MKK polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest as the

30 immunizing antigen. The polypeptide or peptide used to immunize an animal can be derived from translated cDNA or chemically synthesized, and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum

35 albumin and thyroglobulin. The coupled peptide is then

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used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

The invention also features methods of identifying subjects at risk for MKK-mediated disorders by measuring activation of the MKK signal transduction pathway. Activation of the MKK signal transduction pathway can be determined by measuring MKK synthesis; activation of MKK isoforms; activation of MKK substrates p38 or JNK isoforms; or activation of p38 and JNK substrates such as ATF2, ATFa, CRE-BPa, and c-Jun. The term "JNK" or "JNK isoforms" includes both JNK1 and JNK2. The term "MKK substrate" as used herein include MKK substrates, as well as MKK substrate substrates, e.g., p38, JNK, ATF2, and c-Jun.

In one embodiment, activation of the MKK signal transduction pathway is determined by measuring activation of the MKK signal transduction pathway substrates p38, JNK isoforms, ATF2, or c-Jun. MKK activity is measured by the rate of substrate phosphorylation as determined by quantitation of the rate of [³²]P incorporation. The specificity of MKK substrate phosphorylation can be tested by measuring p38 and JNK activation, or by employing mutated p38 and JNK molecules that lack the sites of MKK phosphorylations. Altered phosphorylation of the substrate relative to control values indicates alteration of the MKK signal transduction pathway, and increased risk in a subject of an MKK-mediated disorder. MKK activation of p38 and JNK can be detected in a coupled assay with the MKK signal transduction substrate ATF2, or related compounds such as ATFa and CRE-BPa. Activation can also be detected with the substrate c-Jun. When ATF2 is included in the assay, it is present as an intact protein or as a fragment of the intact protein, e.g., the activation domain (residues 1-109, or a portion thereof). ATF2 is incubated with a

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test sample in which MKK activity is to be measured and [γ - 32 P]ATP, under conditions sufficient to allow the phosphorylation of ATF2. ATF2 is then isolated and the amount of phosphorylation quantitated. In a specific
5 embodiment, ATF2 is isolated by immunoprecipitation, resolved by SDS-PAGE, and detected by autoradiography.

In another embodiment, activation of the MKK signal transduction pathway is determined by measuring the level of MKK expression in a test sample. In a
10 specific embodiment, the level of MKK expression is measured by Western blot analysis. The proteins present in a sample are fractionated by gel electrophoresis, transferred to a membrane, and probed with labeled antibodies to MKK. In another specific embodiment, the
15 level of MKK expression is measured by Northern blot analysis. Polyadenylated [poly(A)⁺] mRNA is isolated from a test sample. The mRNA is fractionated by electrophoresis and transferred to a membrane. The membrane is probed with labeled MKK cDNA. In another
20 embodiment, MKK expression is measured by quantitative PCR applied to expressed mRNA.

The MKKs of the invention are useful to screen reagents that modulate MKK activity. MKKs are activated by phosphorylation. Accordingly, in one aspect, the
25 invention features methods for identifying a reagent which modulates MKK activity, by incubating MKK with the test reagent and measuring the effect of the test reagent on MKK synthesis, phosphorylation, function, or activity. In one embodiment, the test reagent is incubated with MKK
30 and [32]P-ATP, and the rate of MKK phosphorylation determined, as described above. In another embodiment, the test reagent is incubated with a cell transfected with an MKK polynucleotide expression vector, and the effect of the test reagent on MKK transcription is
35 measured by Northern blot analysis, as described above.

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In a further embodiment, the effect of the test reagent on MKK synthesis is measured by Western blot analysis using an antibody to MKK. In still another embodiment, the effect of a reagent on MKK activity is measured by incubating MKK with the test reagent, [³²]P-ATP, and a substrate in the MKK signal transduction pathway, including one or more of p38, JNK, and ATF2. The rate of substrate phosphorylation is determined as described above.

10 The term "modulation of MKK activity" includes inhibitory or stimulatory effects. The invention is particularly useful for screening reagents that inhibit MKK activity. Such reagents are useful for the treatment or prevention of MKK-mediated disorders, for example, inflammation and oxidative damage.

15 The invention further features a method of treating a MKK-mediated disorder by administering to a subject in need thereof an effective dose of a therapeutic reagent that inhibits the activity of MKK.

20 By the term "MKK-mediated disorder" is meant a pathological condition resulting, at least in part, from excessive activation of an MKK signal transduction pathway. The MKK signal transduction pathways are activated by several factors, including inflammation and stress. MKK-mediated disorders include, for example, ischemic heart disease, burns due to heat or radiation (UV, X-ray, γ , β , etc.), kidney failure, liver damage due to oxidative stress or alcohol, respiratory distress syndrome, septic shock, rheumatoid arthritis, autoimmune disorders, and other types of inflammatory diseases.

30 As used herein, the term "therapeutic reagent" means any compound or molecule that achieves the desired effect on an MKK-mediated disorder when administered to a subject in need thereof.

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MKK-mediated disorders further include proliferative disorders, particularly disorders that are stress-related. Examples of stress-related MKK-mediated proliferative disorders are psoriasis, acquired immune deficiency syndrome, malignancies of various tissues of the body, including malignancies of the skin, bone marrow, lung, liver, breast, gastrointestinal system, and genito-urinary tract. Preferably, therapeutic reagents inhibit the activity or expression of MKK inhibit cell growth or cause apoptosis.

A therapeutic reagent that "inhibits MKK activity" interferes with a MKK-mediated signal transduction pathway. For example, a therapeutic reagent can alter the protein kinase activity of MKK, decrease the level of MKK transcription or translation, e.g., an antisense polynucleotide able to bind MKK mRNA, or suppress MKK phosphorylation of p38, JNK, or ATF2, thus disrupting the MKK-mediated signal transduction pathway. Examples of such reagents include antibodies that bind specifically to MKK polypeptides, and fragments of MKK polypeptides that competitively inhibit MKK polypeptide activity.

A therapeutic reagent that "enhances MKK activity" supplements a MKK-mediated signal transduction pathway. Examples of such reagents include the MKK polypeptides themselves, which can be administered in instances where the MKK-mediated disorder is caused by underexpression of the MKK polypeptide. In addition, portions of DNA encoding an MKK polypeptide can be introduced into cells that underexpress an MKK polypeptide.

A "therapeutically effective amount" is an amount of a reagent sufficient to decrease or prevent the symptoms associated with the MKK-mediated disorder.

Therapeutic reagents for treatment of MKK-mediated disorders identified by the method of the invention are administered to a subject in a number of ways known to

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the art, including parenterally by injection, infusion, sustained-release injection or implant, intravenously, intraperitoneally, intramuscularly, subcutaneously, or transdermally. Epidermal disorders and disorders of the
5 epithelial tissues are treated by topical application of the reagent. The reagent is mixed with other compounds to improve stability and efficiency of delivery (e.g., liposomes, preservatives, or dimethyl sulfoxide (DMSO)). Polynucleotide sequences, including antisense sequences,
10 can be therapeutically administered by techniques known to the art resulting in introduction into the cells of a subject suffering from the MKK-mediated disorder. These methods include the use of viral vectors (e.g., retrovirus, adenovirus, vaccinia virus, or herpes virus),
15 colloid dispersions, and liposomes.

The materials of the invention are ideally suited for the preparation of a kit for the detection of the level or activity of MKK. Accordingly, the invention features a kit comprising an antibody that binds MKK, or
20 a nucleic acid probe that hybridizes to a MKK polynucleotide, and suitable buffers. The probe or monoclonal antibody can be labeled to detect binding to a MKK polynucleotide or protein. In a preferred embodiment, the kit features a labeled antibody to MKK.

25 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein
30 can be used in the practice or testing of the present invention, the preferred methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

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Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Detailed Description

5 The drawings will first be described.

Drawings

Fig. 1 is a comparison of the amino acid sequences of MKK3 (SEQ ID NO:2), MKK4- α (SEQ ID NO:6), the human MAP kinase kinases MEK1 (SEQ ID NO:11) and MEK2 (SEQ ID
10 NO:12), and the yeast HOG1 MAP kinase kinase PBS2 (SEQ ID NO:13). MKK3 and MKK4 sequences were compared with the PILE-UP program (version 7.2; Wisconsin Genetics Computer Group). The protein sequences are presented in single letter code [A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G,
15 Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp, and Y, Tyr]. The PBS2 sequence is truncated at both the NH₂- (<) and COOH- (>) termini. Gaps introduced into the sequences to optimize the alignment are illustrated by a
20 dash. Identical residues are indicated by a period. The sites of activating phosphorylation in MEK are indicated by asterisks.

Fig. 2 is a dendrogram showing the relation between members of the human and yeast MAP kinase
25 kinases. The dendrogram was created by the unweighted pair-group method with the use of arithmetic averages (PILE-UP program). The human (hu) MAP kinase kinases MEK1, MEK2, MKK3, and MKK4; the *Saccharomyces cerevisiae* (sc) MAP kinase kinases PBS2, MKK1, and STE7; and the
30 *Saccharomyces pombe* (sp) MAP kinase kinases WIS1 and BYR1 are presented.

Fig. 3 is a schematic representation of the ERK, p38, and JNK signal transduction pathways. MEK1 and MEK2 are activators of th ERK subgroup of MAP kinase. MKK3

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and MKK4 are activators of the p38 MAP kinase. MKK4 is identified as an activator of both the p38 and JNK subgroups of MAP kinase.

Fig. 4 is a representation of the nucleic acid
5 (SEQ ID NO:1) and amino acid sequences (SEQ ID NO:2) for MKK3.

Fig. 5 is a representation of the nucleic acid
(SEQ ID NO:3) and amino acid sequences (SEQ ID NO:4) for MKK6.

10 Fig. 6 is a representation of the nucleic acid
(SEQ ID NO:5) and amino acid sequences (SEQ ID NO:6) for MKK4 α .

Fig. 7 is a representation of the nucleic acid
(SEQ ID NO:7) and amino acid sequences (SEQ ID NO:8) for
15 MKK4 β .

Fig. 8 is a representation of the nucleic acid
(SEQ ID NO:9) and amino acid sequences (SEQ ID NO:10) for MKK4 γ .

Human Mitogen-Activated Protein Kinase Kinases

20 The human MAP kinase kinases MKK3 and MKK4
(MKK3/4), and MKK6 described herein mediate the
transduction of specific signals from the cell surface to
the nucleus along specific pathways. These signal
transduction pathways are initiated by factors such as
25 cytokines, UV radiation, osmotic shock, and oxidative
stress. Activation of MKK3/4 results in activation of
the MAP kinases p38 (MKK3/4) and JNK (MKK4). p38 and JNK
in turn activate a group of related transcription factors
such as ATF2, ATFa, and CRE-BPa. These transcription
30 factors in turn activate expression of specific genes.
For example, ATF2 is known to activate expression of
human T cell leukemia virus 1 (Wagner and Green (1993)
Science 262:395), transforming growth factor-b2 (Kim et
al. (1992) supra), interferon- β (Du et al. (1993) Cell

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74:887), and E-selectin (DeLuca et al. (1994) J. Biol. Chem. 269:19193). In addition, ATF2 is implicated in the function of a T cell-specific enhancer (Georgopoulos et al. (1992) Mol. Cell. Biol. 12:747).

5 The isolation of human MKKs is described in Example 1 and in Dérijard et al. (1995) Science 267:682-685. Distinctive regions of the yeast PBS2 sequence were used to design polymerase chain reaction (PCR) primers. Amplification of human brain mRNA with these primers
10 resulted in the formation of specific products which were cloned into a plasmid vector and sequenced. Two different complementary DNAs (cDNAs) that encoded human protein kinases were identified: one encoding a 36 kD protein (MKK3), and one encoding a 44 kD protein (MKK4).
15 MKK4 includes 3 isoforms that vary slightly at the NH₂-terminal, identified as α , β , and γ . The amino acid sequences of MKK3 (SEQ ID NO:2), MKK4- α (SEQ ID NO:6), MKK4- β (SEQ ID NO:8), and MKK4- γ (SEQ ID NO:10) are shown in Fig. 1. The nucleic acid and amino acid sequences of
20 MKK3 (Fig. 5), MKK6 (Fig. 6), MKK4 α (Fig. 7), MKK4 β (Fig. 8), and MKK4 γ (Fig. 9) are also provided. MKK6 was isolated from a human skeletal muscle library by cross-hybridization with MKK3. Except for differences at the N-terminus, MKK6 is homologous to MKK3. Other human MKK3
25 and MKK4 isoforms that exist can be identified by the method described in Example 1.

 The expression of these human MKK isoforms was examined by Northern (RNA) blot analysis of mRNA isolated from eight adult human tissues (Example 2). Both protein
30 kinases were found to be widely expressed in human tissues, with the highest expression seen in skeletal muscle tissue.

 The substrate specificity of MKK3 was investigated in an *in vitro* phosphorylation assay with recombinant
35 epitope-tagged MAP kinases (JNK1, p38, and ERK2) as

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substrates (Example 3). MKK3 and MKK6 phosphorylated p38, but did not phosphorylate JNK1 or ERK2.

Phosphoaminoacid analysis of p38 demonstrated the presence of a phosphothreonine and phosphotyrosine.

5 Mutational analysis of p38 demonstrated that replacement of phosphorylation sites Thr¹⁸⁰ and Tyr¹⁸² with Ala and Phe, respectively, blocked p38 phosphorylation. These results establish that MKK3 functions *in vitro* as a p38 MAP kinase kinase. The substrate specificity of MKK6 is
10 similar to that of MKK3, but the specific activity of MKK6 is approximately 300-fold greater than that of MKK3.

Studies of the *in vitro* substrate specificity of MKK4 are described in Example 4. MKK4 incubated with [γ -³²P]ATP, and JNK1, p38, or ERK2 was found to phosphorylate
15 both p38 and JNK1. MKK4 activation of JNK and p38 was also studied by incubating MKK4 with wild-type or mutated JNK1 or p38. The p38 substrate ATF2 was included in each assay. MKK4 was found to exhibit less autophosphorylation than MKK3. MKK4 was also found to be
20 a substrate for activated MAP kinase. Unlike MKK3 and MKK6, MKK4 was also found to activate JNK1. MKK4 incubated with wild-type JNK1, but not mutated JNK1, resulted in increased phosphorylation of ATF2. These results establish that MKK4 is a p38 MAP kinase kinase
25 that also phosphorylates the JNK subgroup of MAP kinases.

In vivo activation of p38 by UV-stimulated MKK3 is described in Example 5. Cells expressing MKK3 were exposed in the presence or absence of UV radiation. MKK3 was isolated by immunoprecipitation and used for protein
30 kinase assays with the substrates p38 or JNK. ATF2 was included in some assays as a substrate for p38 and JNK. MKK3 from non-activated cultured COS cells caused a small amount of phosphorylation of p38 MAP kinase, resulting from basal activity of MKK3. MKK3 from UV-irradiated
35 cells caused increased phosphorylation of p38 MAP kinase,

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but not of JNK1. An increase in p38 activity was also detected in assays in which ATF2 was included as a substrate. These results establish that MKK3 is activated by UV radiation.

5 The effect of expression of MKK3 and MKK4 on p38 activity was examined in COS-1 cells (Example 6). Cells were transfected with a vector encoding p38 and a MEK1, MKK3, or MKK4. Some of the cells were also exposed to EGF or UV radiation. p38 was isolated by
10 immunoprecipitation and assayed for activity with [γ -³²P]ATP and ATF2. The expression of the ERK activator MEK1 did not alter p38 phosphorylation of ATF2. In contrast, expression of MKK3 or MKK4 caused increased activity of p38 MAP kinase. The activation of p38 caused
15 by MKK3 and MKK4 was similar to that observed in UV-irradiated cells, and was much greater than that detected in EGF-treated cells. These *in vitro* results provide evidence that MKK3 and MKK4 activate p38 *in vivo*.

A series of experiments was conducted to examine
20 the potential regulation of ATF2 by JNK1. These experiments are described in Gupta et al. (1995) Science 267:389-393. The effect of UV radiation on ATF2 phosphorylation was investigated in COS-1 cells transfected with and without epitope-tagged JNK1 (Example
25 7). Cells were exposed to UV radiation, and JNK1 and JNK2 visualized by in-gel protein kinase assay with the substrate ATF2. JNK1 and JNK2 were detected in transfected and non-transfected cells exposed to UV radiation; however, JNK1 levels were higher in the
30 transfected cells. These results demonstrate that ATF2 is a substrate for the JNK1 and JNK2 protein kinases, and that these protein kinases are activated in cells exposed to UV light.

The site of JNK1 phosphorylation of ATF2 was
35 examined by deletion analysis (Example 8). Progressive

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NH₂-terminal domain deletion GST-ATF2 fusion proteins were generated, and phosphorylation by JNK1 isolated from UV-irradiated cells was examined. The results showed that JNK1 requires the presence of ATF2 residues 1-60 for phosphorylation of the NH₂-terminal domain of ATF2.

The ATF2 residues required for binding of JNK1 were similarly examined. JNK1 was incubated with immobilized ATF2, unbound JNK1 was removed by extensive washing, and bound JNK1 was detected by incubation with [γ-³²P]ATP. Results indicate that residues 20 to 60 of ATF2 are required for binding and phosphorylation by JNK1. A similar binding interaction between ATF2 and the 55 kD JNK2 protein kinase has also been observed.

Phosphorylation by JNK1 was shown to reduce the electrophoretic mobility of ATF2 (Example 9). Phosphoamino acid analysis of the full-length ATF2 molecule (residues 1-505) demonstrated that JNK phosphorylated both Thr and Ser residues. The major sites of Thr and Ser phosphorylation were located in the NH₂ and COOH terminal domains, respectively. The NH₂-terminal sites of phosphorylation were identified as Thr⁶⁹ and Thr⁷¹ by phosphopeptide mapping and mutational analysis. These sites of Thr phosphorylation are located in a region of ATF2 that is distinct from the sub-domain required for JNK binding (residues 20 to 60).

The reduced electrophoretic mobility seen with phosphorylation of ATF2 was investigated further (Example 10). JNK1 was activated in CHO cells expressing JNK1 by treatment with UV radiation, pro-inflammatory cytokine interleukin-1 (IL-1), or serum. A decreased electrophoretic mobility of JNK1-activated ATF2 was observed in cells treated with UV radiation and IL-1. Smaller effects were seen after treatment of cells with serum. These results indicate that ATF2 is an *in vivo* substrate for JNK1.

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The effect of UV radiation on the properties of wild-type (Thr^{69,71}) and phosphorylation-defective (Ala^{69,71}) ATF2 molecules was investigated (Example 11). Exposure to UV caused a decrease in the electrophoretic mobility of both endogenous and over-expressed wild-type ATF2. This change in electrophoretic mobility was associated with increased ATF2 phosphorylation. Both the electrophoretic mobility shift and increased phosphorylation were blocked by the replacement of Thr⁶⁹ and Thr⁷¹ with Ala in ATF2. This mutation also blocked the phosphorylation of ATF2 on Thr residues *in vivo*.

Transcriptional activities of fusion proteins consisting of the GAL4 DNA binding domain and wild-type or mutant ATF2 were examined (Example 12). Point mutations at Thr⁶⁹ and/or Thr⁷¹ of ATF2 significantly decreased the transcriptional activity of ATF2 relative to the wild-type molecule, indicating the physiological relevance of phosphorylation at these sites for activity.

The binding of JNK1 to the NH₂-terminal activation domain of ATF2 (described in Example 8) suggested that a catalytically inactive JNK1 molecule could function as a dominant inhibitor of the wild-type JNK1 molecule. This hypothesis was investigated by examining the effect of a catalytically inactive JNK1 molecule on ATF2 function (Example 13). A catalytically-inactive JNK1 mutant was constructed by replacing the sites of activating Thr¹⁸³ and Tyr¹⁸⁵ phosphorylation with Ala and Phe, respectively (Ala¹⁸³, Phe¹⁸⁵, termed "dominant-negative"). Expression of wild-type JNK1 caused a small increase in serum-stimulated ATF2 transcriptional activity. In contrast, dominant-negative JNK1 inhibited both control and serum-stimulated ATF2 activity. This inhibitory effect results from the non-productive binding of the JNK1 mutant to the ATF2 activation domain, effectively blocking ATF2 phosphorylation.

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The tumor suppressor gene product Rb binds to ATF2 and increases ATF2-stimulated gene expression (Kim et al. (1992) Nature 358:331). Similarly, the adenovirus oncoprotein E1A associates with the DNA binding domain of ATF2 and increases ATF2-stimulated gene expression by a mechanism that requires the NH₂-terminal activation domain of ATF2 (Liu and Green (1994) Nature 368:520). ATF2 transcriptional activity was investigated with the luciferase reporter gene system in control, Rb-treated, and E1A-treated cells expressing wild-type or mutant ATF2 molecules (Example 14). Rb and E1A were found to increase ATF2-stimulated gene expression of both wild-type and mutant ATF2. However, mutant ATF2 caused a lower level of reporter gene expression than did wild-type ATF2. Together, these results indicate a requirement for ATF2 phosphorylation (on Thr⁶⁹ and Thr⁷¹) plus either Rb or E1A for maximal transcriptional activity. Thus, Rb and E1A act in concert with ATF2 phosphorylation to control transcriptional activity.

A series of experiments were conducted to examine the action of p38 activation and to establish the relationship of the p38 MAP kinase pathway to the ERK and JNK signal transduction pathways (Raingeaud et al. (1995) J. Biol. Chem. 270:7420). Initially, the substrate specificity of p38 was investigated by incubating p38 with proteins that have been demonstrated to be substrates for the ERK and/or JNK groups of MAP kinases (Example 15). We examined the phosphorylation of MBP (Erickson et al. (1990) J. Biol. Chem. 265:19728), EGF-R (Northwood et al. (1991) J. Biol. Chem. 266:15266), cytoplasmic phospholipase A₂ (cPLA₂) (Lin et al. (1993) Cell 72:269), c-Myc (Alvarez et al. (1991) J. Biol. Chem. 266:15277), IκB, c-Jun, and wild-type (Thr^{69,71}) or mutated (Ala^{69,71}) ATF2. p38 phosphorylated MBP and EGF-R, and to a lesser extent IκB, but not the other ERK

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substrates, demonstrating that the substrate specificity of p38 differs from both the ERK and JNK groups of MAP kinases. Wild-type ATF2, but not mutated ATF2 (Ala^{69,71}), was found to be an excellent p38 substrate.

- 5 The phosphorylation of ATF2 by p38 was associated with an electrophoretic mobility shift of ATF2 during polyacrylamide gel electrophoresis. We tested the hypothesis that p38 phosphorylates ATF2 at the same sites as JNK1 by replacing Thr⁶⁹ and Thr⁷¹ with Ala (Ala^{69,71}).
- 10 It was found that p38 did not phosphorylate mutated ATF2, which demonstrates that p38 phosphorylates ATF2 within the NH₂-terminal activation domain on Thr⁶⁹ and Thr⁷¹.

- A comparison of the binding of JNK and p38 to ATF2 was conducted by incubating extracts of cells expressing
- 15 JNK1 or p38 with epitope alone (GST) or GST-ATF2 (residues 1-109 containing the activation domain) (Example 16). Bound protein kinases were detected by Western blot analysis. The results demonstrate that both p38 and JNK bind to the ATF2 activation domain.

- 20 EGF and phorbol ester are potent activators of the ERK signal transduction pathway (Egan and Weinberg (1993) Nature 365:781), causing maximal activation of the ERK sub-group of MAP kinases. These treatments, however, cause only a small increase in JNK protein kinase
- 25 activity (Dérillard et al. (1994) supra; Hibi et al. (1993) supra). The effects of EGF or phorbol esters, as well UV radiation, osmotic shock, interleukin-1, tumor necrosis factor, and LPS, on p38 activity were all tested (Example 17). Significantly, EGF and phorbol ester
- 30 caused only a modest increase in p38 protein kinase activity, whereas environmental stress (UV radiation and osmotic shock) caused a marked increase in the activity of both p38 and JNK. Both p38 and JNK were activated in cells treated with pro-inflammatory cytokines (TNF and
- 35 IL-1) or endotoxic LPS. Together, these results indicate

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that p38, like JNK, is activated by a stress-induced signal transduction pathway.

ERKs and JNKs are activated by dual phosphorylation within the motifs Thr-Glu-Tyr and Thr-Pro-Tyr, respectively. In contrast, p38 contains the related sequence Thr-Gly-Tyr. To test whether this motif is relevant to the activation of p38, the effect of the replacement of Thr-Gly-Tyr with Ala-Gly-Phe was examined (Example 18). The effect of UV radiation on cells expressing wild-type (Thr¹⁸⁰, Tyr¹⁸²) or mutant p38 (Ala¹⁸⁰, Phe¹⁸²) was studied. Western blot analysis using an anti-phosphotyrosine antibody demonstrated that exposure to UV radiation caused an increase in the Tyr phosphorylation of p38. The increased Tyr phosphorylation was confirmed by phosphoaminoacid analysis of p38 isolated from [γ -³²P]phosphate-labeled cells. This analysis also demonstrated that UV radiation caused increased Thr phosphorylation of p38. Significantly, the increased phosphorylation on Thr¹⁸⁰ and Tyr¹⁸² was blocked by the Ala¹⁸⁰/Phe¹⁸² mutation. This result demonstrates that UV radiation causes increased activation of p38 by dual phosphorylation.

It has recently been demonstrated that ERK activity is regulated by the mitogen-induced dual specificity phosphatases MKP1 and PAC1 (Ward et al. (1994) Nature 367:651). The activation of p38 by dual phosphorylation (Example 18) raises the possibility that p38 may also be regulated by dual specificity phosphatases. We examined the effect of MKP1 and PAC1 on p38 MAP kinase activation (Example 19). Cells expressing human MKP1 and PAC1 were treated with and without UV radiation, and p38 activity measured. The expression of PAC1 or MKP1 was found to inhibit p38 activity. The inhibitory effect of MKP1 was greater than PAC1. In contrast, cells transfected with a catalytically inactive

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mutant phosphatase (mutant PAC1 Cys²⁵⁷/Ser) did not inhibit p38 MAP kinase. These results demonstrate that p38 can be regulated by dual specificity phosphatases PAC1 and MKP1.

5 The sub-cellular distribution of p38 MAP kinase was examined by indirect immunofluorescence microscopy (Example 20). Epitope-tagged p38 MAP kinase was detected using the M2 monoclonal antibody. Specific staining of cells transfected with epitope-tagged p38 MAP kinase was
10 observed at the cell surface, in the cytoplasm, and in the nucleus. Marked changes in cell surface and nuclear p38 MAP kinase were not observed following UV irradiation, but an increase in the localization of cytoplasmic p38 MAP kinase to the perinuclear region was
15 detected.

A series of experiments were conducted to study the activation of JNK by hyper-osmotic media (Example 21). These experiments were reported by Galcheva-Gargova et al. (1994) Science 265:806. CHO cells expressing
20 epitope-tagged JNK1 were incubated with 0 - 1000 mM sorbitol, and JNK1 activity measured in an immune complex kinase assay with the substrate c-Jun. Increased JNK1 activity was observed in cells incubated 1 hour with 100 mM sorbitol. Increased JNK1 activity was observed within
25 5 minutes of exposure to 300 mM sorbitol. Maximal activity was observed 15 to 30 minutes after osmotic shock with a progressive decline in JNK1 activity at later times. The activation of JNK by osmotic shock was studied in cells expressing wild-type (Thr¹⁸³, Tyr¹⁸⁵) or
30 mutated (Ala¹⁸³, Phe¹⁸⁵) JNK1. JNK1 activity was measured after incubation for 15 minutes with or without 300 mM sorbitol. Cells expressing wild-type JNK1 showed increased JNK1 activity, while cells expressing mutated JNK1 did not. These results demonstrate that the JNK

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signal transduction pathway is activated in cultured mammalian cells exposed to hyper-osmotic media.

The results of the above-described experiments are illustrated in Fig. 3, which diagrams the ERK, p38, and JNK MAP kinase signal transduction pathways. ERKs are potentially activated by treatment of cells with EGF or phorbol esters. In contrast, p38 is only slightly activated under these conditions (Example 15). However, UV radiation, osmotic stress, and inflammatory cytokines cause a marked increase in p38 activity. This difference in the pattern of activation of ERK and p38 suggests that these MAP kinases are regulated by different signal transduction pathways. The molecular basis for the separate identity of these signal transduction pathways is established by the demonstration that the MAP kinase kinases that activate ERK (MEK1 and MEK2) and p38 (MKK3, MKK6, and MKK4) are distinct.

MKK isoforms are useful for screening reagents which modulate MKK activity. Described in the Use section following the examples are methods for identifying reagents capable of inhibiting or activating MKK activity.

The discovery of human MKK isoforms and MKK-mediated signal transduction pathways is clinically significant for the treatment of MKK-mediated disorders. One use of the MKK isoforms is in a method for screening reagents able to inhibit or prevent the activation of the MKK-MAP kinase- ATF2 pathways.

The following examples are meant to illustrate, not limit, the invention.

Example 1. MKK Protein Kinases

The primary sequences of MKK3 and MKK4 were deduced from the sequence of cDNA clones isolated from a human fetal brain library.

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- The primers TTYTAYGGNGCNTTYTTYATHGA (SEQ ID NO:14) and ATBCTYTCNGGNGCCATKTA (SEQ ID NO:15) were designed based on the sequence of PBS2 (Brewster et al. (1993) Science 259:1760; Maeda et al. (1994) Nature 369:242).
- 5 The primers were used in a PCR reaction with human brain mRNA as template. Two sequences that encoded fragments of PBS2-related protein kinases were identified. Full-length human cDNA clones were isolated by screening of a human fetal brain library (Dérillard et al. (1994) supra).
- 10 The cDNA clones were examined by sequencing with an Applied Biosystems model 373A machine. The largest clones obtained for MKK3 (2030 base pairs (bp)) and MKK4 (3576 bp) contained the entire coding region of these protein kinases.
- 15 The primary structures of MKK3 (SEQ ID NO:2) and MKK4 α (SEQ ID NO:6) are shown in Fig. 1. An in-frame termination codon is located in the 5' untranslated region of the MKK3 cDNA, but not in the 5' region of the MKK4 cDNA. The MKK4 protein sequence presented starts at
- 20 the second in-frame initiation codon.
- These sequences were compared to those of the human MAP kinase kinases MEK1 (SEQ ID NO:11) and MEK2 (SEQ ID NO:12) (Zheng and Guan (1993) J. Biol. Chem 268:11435) and of the yeast MAP kinase kinase PBS2 (SEQ
- 25 ID NO:13) (Boguslawski and Polazzi (1987) Proc. Natl. Acad. Sci. USA 84:5848) (Fig. 1). The identity and similarity of the kinases with human MKK3 (between subdomains I and XI) were calculated with the BESTFIT program (version 7.2; Wisconsin Genetics Computer Group)
- 30 (percent of identity to percent of similarity): MEK1, 41%/63%; MEK2, 41%/62%; MKK4 α , 52%/73%; and PBS2, 40%/59%). The identity and similarity of the kinases with human MKK4 α were calculated to be as follows (percent of identity to percent of similarity): MEK1,
- 35 44%/63%; MEK2, 45%/61%; MKK3, 52%/73%; and PBS2, 44%/58%.

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The cDNA sequences of MKK3 and MKK4 γ have been deposited in GenBank with accession numbers L36719 and L36870, respectively. The MKK4 γ cDNA sequence contains both the cDNA sequences of MKK4 α and MKK4 β , which are
5 generated in vivo from alternate splicing sites. One of ordinary skill in the art can readily determine the amino acid sequences of MKK3 and MKK4 isoforms from the deposited cDNA sequences.

Human MKK6 cDNA clones were isolated from a
10 skeletal muscle library by screening with an MKK3 probe at low stringency. Mammalian MKK6 expression vectors were constructed by sub-cloning the MKK6 cDNA in the *HindIII* and *XbaI* sites of pCDNA3 (Invitrogen Inc.). The sequences of all plasmids were confirmed by automated
15 sequencing with an Applied Biosystems model 373A machine.

Example 2. Expression of MKK3 and MKK4 mRNA in Adult Human Tissue

Northern blot analysis was performed with polyadenylated [poly(A)⁺] mRNA (2 μ g) isolated from human
20 heart, brain, placenta, lung, liver, muscle, kidney, and pancreas tissues. The mRNA was fractionated by denaturing agarose gel electrophoresis and was transferred to a nylon membrane. The blot was probed with the MKK3 and MKK4 cDNA labeled by random priming
25 with [α -³²P]ATP (deoxyadenosine triphosphate) (Amersham International PLC). MKK3 and MKK4 were expressed in all tissues examined; the highest expression of MKK3 and MKK4 was found in skeletal muscle tissue.

The relation between members of the human and
30 yeast MAP kinase kinase group is presented as a dendrogram (Fig. 2). MKK3/4 form a unique subgroup of human MAP kinase kinases.

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Example 3. In Vitro Phosphorylation of p38 MAP kinase by MKK3

GST-JNK1, and GST-ERK2 have been described (Dérillard et al. (1994) supra; Gupta et al. (1995) Science 267:389; Wartmann and Davis (1994) J. Biol. Chem. 269:6695). GST-p38 MAP kinase was prepared from the expression vector pGSTag (Dressier et al. (1992) Biotechniques 13:866) and a PCR fragment containing the coding region of the p38 MAP kinase cDNA. GST-MKK3 and MKK4 were prepared with pGEX3X (Pharmacia-LKB Biotechnology) and PCR fragments containing the coding region of the MKK3 and MKK4 cDNAs. The GST fusion proteins were purified by affinity chromatography with the use of GSH-agarose (Smith and Johnson (1988) Gene 67:31). The expression vectors pCMV-Flag-JNK1 and pCMV-MEK1 have been described (Dérillard et al. (1994) supra; Wartmann and Davis (1994) supra). The plasmid pCMV-Flag-p38 MAP kinase was prepared with the expression vector pCMV5 (Andersson et al. (1989) J. Biol. Chem. 264:8222) and the p38 MAP kinase cDNA. The expression vectors for MKK3 and MKK4 were prepared by subcloning of the cDNAs into the polylinker of pCDNA3 (Invitrogen). The Flag epitope (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQ ID NO:16); Immunex, Seattle, WA) was inserted between codons 1 and 2 of the kinases by insertional overlapping PCR (Ho et al. (1989) Gene 77:51).

Protein kinase assays were performed in kinase buffer (25 mM 4-(2-hydroxyethyl)-1-piperazineethansulfonic acid, pH 7.4, 25 mM β -glycerophosphate, 25 mM $MgCl_2$, 2 mM dithiothreitol, and 0.1 mM orthovanadate). Recombinant GST-MKK3 was incubated with $[\gamma\text{-}^{32}P]\text{ATP}$ and buffer, GST-JNK1, GST-p38 MAP kinase, or GST-ERK2. The assays were initiated by the addition of 1 μg of substrate proteins and 50 μM $[\gamma\text{-}^{32}P]\text{ATP}$ (10 Ci/mmol) in a final volume of 25 μl . The

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reactions were terminated after 30 minutes at 25°C by addition of Laemmli sample buffer. The phosphorylation of the substrate proteins was examined after SDS-polyacrylamide gel electrophoresis (SDS-PAGE) by
5 autoradiography. Phosphoaminoacid analysis was performed by partial acid hydrolysis and thin-layer chromatography (Dérijard et al. (1994) supra; Alvarez et al. (1991) J. Biol. Chem. 266:15277). Autophosphorylation of MKK3 was observed in all groups. MKK3 phosphorylated p38 MAP
10 kinase, but not JNK1 or ERK2.

A similar insertional overlapping PCR procedure was used to replace Thr¹⁸⁰ and Tyr¹⁸² of p38, with Ala and Phe, respectively. The sequence of all plasmids was confirmed by automated sequencing on an Applied
15 Biosystems model 373A machine. GST-MKK3 was incubated with [γ -³²P]ATP and buffer, wild-type GST-p38 MAP kinase (TGY), or mutated GST-p38 MAP kinase (AGF). The phosphorylated proteins were resolved by SDS-PAGE and detected by autoradiography. Only phosphorylation of
20 wild-type p38 was observed.

MKK6 was similarly tested and shown to phosphorylate p38 MAP kinase on Thr¹⁸⁰ and Tyr¹⁸², but not JNK1 or ERK2. The specific activity of MKK6 was approximately 300-fold greater than that of MKK3.

25 Example 4. In Vitro Phosphorylation and Activation of JNK and p38 MAP Kinase by MKK4

Protein kinase assays were conducted as described in Example 3. Recombinant GST-MKK4 was incubated with [γ -³²P]ATP and buffer, GST-JNK1, GST-p38 MAP kinase, or
30 GST-ERK2. JNK1 and p38 were phosphorylated, as was MKK4 incubated with JNK1 and p38.

GST-MKK4 was incubated with [γ -³²P]ATP and buffer, wild-type JNK1 (Thr¹⁸³, Tyr¹⁸⁵), or mutated GST-JNK1 (Ala¹⁸³, Phe¹⁸⁵). The JNK1 substrate ATF2 (Gupta et al.

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(1995) supra) was included in each incubation. ATF2 was phosphorylated in the presence of MKK4 and wild-type JNK1. The results establish that MKK4 phosphorylates and activates both p38 and JNK1.

5 Example 5. Phosphorylation and Activation of p38 MAP Kinase by UV-stimulated MKK3

Epitope-tagged MKK3 was expressed in COS-1 cells maintained in Dulbecco's modified Eagle's medium supplemented with fetal bovine serum (5%) (Gibco-BRL).

- 10 The cells were transfected with the lipofectamine reagent according to the manufacturer's recommendations (Gibco-BRL) and treated with UV radiation or EGF as described (Dérillard et al. (1994) supra).

The cells were exposed in the absence and presence
15 of UV-C (40 J/m²). The cells were solubilized with lysis buffer (20 mM tris, pH 7.4, 1% Triton X-100, 10% glycerol, 137 mM NaCl, 2 mM EDTA, 25 mM β -glycerophosphate, 1 mM Na orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and leupeptin (10 μ g/ml))
20 and centrifuged at 100,000 x g for 15 minutes at 4°C. MKK3 was isolated by immunoprecipitation. The epitope-tagged protein kinases were incubated for 1 hour at 4°C with the M2 antibody to the Flag epitope (IBI-Kodak) bound to protein G-Sepharose (Pharmacia-LKB
25 Biotechnology). The immunoprecipitates were washed twice with lysis buffer and twice with kinase buffer.

Protein kinase assays were conducted with the substrate GST-p38 MAP kinase or JNK1. ATF2 was included in some assays. Basal levels of MKK3 phosphorylation of
30 p38 MAP kinase were observed. UV-irradiation resulted in increased phosphorylation of p38 MAP kinase, but not of JNK1. The increased p38 MAP kinase activity resulted in increased phosphorylation of ATF2.

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Example 6. Activation of p38 MAP Kinase in Cells
Expressing MKK3 and MKK4

COS-1 cells were transfected with epitope-tagged p38 MAP kinase, together with an empty expression vector or an expression vector encoding MEK1, MKK3, or MKK4 α . Some of the cultures were exposed to UV radiation (40 J/m²) or treated with 10 nM EGF. p38 MAP kinase was isolated by immunoprecipitation with M2 monoclonal antibody, and the protein kinase activity was measured in the immunocomplex with [γ -³²P]ATP and ATF2 as substrates. The product of the phosphorylation reaction was visualized after SDS-PAGE by autoradiography. ATF2 was not phosphorylated in the control MEK1, or EGF-treated groups, but was phosphorylated in the MKK3, MKK4, and UV-irradiated groups. MKK3 and MKK4 phosphorylation of ATF2 was similar to that seen with p38 MAP kinase isolated from UV-irradiated cells.

Example 7. Phosphorylation of ATF2 by JNK1 and JNK2

COS-1 cells were maintained in Dulbecco's modified Eagle's medium supplemented with bovine serum albumin (5%) (Gibco-BRL). Metabolic labeling with [³²]P was performed by incubation of cells for 3 hours in phosphate-free modified Eagle's medium (Flow Laboratories Inc.) supplemented with [³²P]orthophosphate (2 mCi/ml) (Dupont-NEN). COS-1 cells were transfected without (Mock) and with epitope-tagged JNK1 (JNK1). Plasmid expression vectors encoding the JNK1 cDNA have previously been described (Dérizard et al. (1994) Cell 76:1025). Plasmid DNA was transfected into COS-1 cells by the lipofectamine method (Gibco-BRL). After 48 hours of incubation, some cultures were exposed to 40 J/m² UV radiation and incubated for 1 hour at 37°C.

Cells were lysed in 20 mM Tris, pH 7.5, 25 mM β -glycerophosphate, 10% glycerol, 1% Triton X-100, 0.5%

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(w/v) deoxycholate, 0.1% (w/v) SDS, 0.137 M NaCl, 2 mM pyrophosphate, 1 mM orthovanadate, 2 mM EDTA, 10 µg/ml leupeptin, 1 mM PMSF. Soluble extracts were prepared by centrifugation in a microfuge for 20 minutes at 4°C.

5 JNK1 immunoprecipitates were also prepared by reaction with a rabbit antiserum prepared with recombinant JNK1 as an antigen.

In-gel protein kinase assays were performed with cell lysates and JNK1 immunoprecipitates after SDS-PAGE
10 by renaturation of protein kinases, polymerization of the substrate (GST-ATF2, residues 1-505) in the gel, and incubation with [γ -³²P]ATP (Dérjard et al. (1994) supra). The incorporation of [³²P]phosphate was visualized by autoradiography and quantitated with a Phosphorimager and
15 ImageQuant soft-ware (Molecular Dynamics Inc., Sunnyvale, CA). The cell lysates demonstrate the presence of 46 kD and 55 kD protein kinases that phosphorylate ATF2 in extracts prepared from UV-irradiated cells. The 46 kD and 55 kD protein kinases were identified as JNK1 and
20 JNK2, respectively.

Example 8. Binding of JNK1 to ATF2 and Phosphorylation of the NH₂-Terminal Activation Domain

The site of JNK1 phosphorylation of ATF2 was investigated by generation of progressive NH₂-terminal
25 domain deletions of ATF2. Plasmid expression vectors encoding ATF2 (pECE-ATF2) (Liu and Green (1994) and (1990)), have been described. Bacterial expression vectors for GST-ATF2 fusion proteins were constructed by sub-cloning ATF2 cDNA fragments from a polymerase chain
30 reaction (PCR) into pGEX-3X (Pharmacia-LKB Biotechnology Inc.). The sequence of all constructed plasmids was confirmed by automated sequencing with an Applied Biosystems model 373A machine. The GST-ATF2 proteins were purified as described (Smith and Johnson (1988) Gene

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67:31), resolved by SDS-PAGE and stained with Coomassie blue. GST-ATF2 fusion proteins contained residues 1-505, 1-349, 350-505, 1-109, 20-109, 40-109, and 60-109.

The phosphorylation of GST-ATF2 fusion proteins by JNK1 isolated from UV-irradiated cells was examined in an immunocomplex kinase assay. Immunocomplex kinase assays were performed with Flag epitope-tagged JNK1 and the monoclonal antibody M2 (IBI-Kodak) as described by Dérillard et al. (1994) supra. Immunocomplex protein kinase assays were also performed with a rabbit antiserum prepared with recombinant JNK1 as an antigen. The cells were solubilized with 20 mM Tris, pH 7.5, 10% glycerol, 1% Triton X-100, 0.137 M NaCl, 25 mM β -glycerophosphate, 2 mM EDTA, 1 mM orthovanadate, 2 mM pyrophosphate, 10 μ g/ml leupeptin, and 1 mM PMSF. JNK1 was immunoprecipitated with protein G-Sepharose bound to a rabbit polyclonal antibody to JNK or the M2 monoclonal antibody to the Flag epitope. The beads were washed three times with lysis buffer and once with kinase buffer (20 mM Hepes, pH 7.6, 20 mM $MgCl_2$, 25 mM β -glycerophosphate, 100 μ M Na orthovanadate, 2 mM dithiothreitol). The kinase assays were performed at 25°C for 10 minutes with 1 μ g of substrate, 20 μ M adenosine triphosphate and 10 μ Ci of [γ - ^{32}P]ATP in 30 μ l of kinase buffer. The reactions were terminated with Laemmli sample buffer and the products were resolved by SDS-PAGE (10% gel). JNK1 phosphorylates GST-ATF2 fusion proteins containing residues 1-505, 1-349, 1-109, 20-109, and 40-109, but not 60-109. These results indicate that the presence of ATF2 residues 1-60 are required for phosphorylation by JNK.

The binding of immobilized GST-ATF2 fusion proteins was examined in a solid-phase kinase assay as described by Hibi et al. (1993) *Genes Dev.* 7:2135. JNK1 from UV-irradiated cells was incubated with GST-ATF2

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fusion proteins bound to GSH-agarose. The agarose beads were washed extensively to remove the unbound JNK1. Phosphorylation of the GST-ATF2 fusion proteins by the bound JNK1 protein kinase was examined by addition of [γ -³²P]ATP. JNK1 bound GST-ATF2 fusion proteins containing residues 1-505, 1-349, 1-109, 20-109, and 40-109, indicating that the presence of residues 20-60 were required for binding of JNK1 to ATF2.

Example 9. Phosphorylation of the NH₂-terminal
10 Activation Domain of ATF2 on Thr⁶⁹ and Thr⁷¹
by JNK1

The effect of UV radiation on the properties of wild-type (Thr^{69,71}) and phosphorylation-defective (Ala^{69,71}) ATF2 molecules was examined. Mock-transfected and JNK1-transfected COS cells were treated without and with 40 J/m² UV radiation. The epitope-tagged JNK1 was isolated by immunoprecipitation with the M2 monoclonal antibody. The phosphorylation of GST-ATF2 (residues 1 to 109) was examined in an immunocomplex kinase assay as described above. The GST-ATF2 was resolved from other proteins by SDS-PAGE and stained with Coomassie blue. The phosphorylation of GST-ATF2 was detected by autoradiography. JNK1-transfected cells, but not mock-transfected cells, phosphorylated ATF2. JNK1 phosphorylation of ATF2 was greater in cells exposed to UV radiation. Phosphorylation of ATF2 by JNK1 was associated with a decreased electrophoretic mobility.

In a separate experiment, GST fusion proteins containing full-length ATF2 (residues 1 to 505), an NH₂-terminal fragment (residues 1 to 109), and a COOH-terminal fragment (residues 95 to 505) were phosphorylated with JNK1 and the sites of phosphorylation analyzed by phosphoamino acid analysis. The methods used for phosphopeptide mapping and phosphoamino acid analysis

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have been described (Alvarez et al. (1991) J. Biol. Chem. 266:15277). The horizontal dimension of the peptide maps was electrophoresis and the vertical dimension was chromatography. The NH₂-terminal sites of phosphorylation were identified as Thr⁶⁹ and Thr⁷¹ by phosphopeptide mapping and mutational analysis. Site-directed mutagenesis was performed as described above, replacing Thr⁶⁹ and Thr⁷¹ with Ala. Phosphorylation of mutated ATF2 was not observed.

10 Example 10. Reduced Electrophoretic Mobility of JNK-Activated ATF2

CHO cells were maintained in Ham's F12 medium supplemented with 5% bovine serum albumin (Gibco-BRL). Cells were labeled and transfected with JNK1 as described above. CHO cells were treated with UV-C (40 J/m²), IL-1 α (10 ng/ml) (Genzyme), or fetal bovine serum (20%) (Gibco-BRL). The cells were incubated for 30 minutes at 37°C prior to harvesting. The electrophoretic mobility of ATF2 after SDS-PAGE was examined by protein immuno-blot analysis. A shift in ATF2 electrophoretic mobility was observed in cells treated with UV, IL-1, and serum. These results indicate that JNK1 activation is associated with an electrophoretic mobility shift of ATF2, further suggesting that ATF2 is an *in vivo* substrate for JNK1.

25 Example 11. Increased ATF2 Phosphorylation After Activation of JNK

COS-1 cells were transfected without (control) and with an ATF2 expression vector (ATF2), as described above (Hai et al. (1989) *supra*). The effect of exposure of the cells to 40 J/m² UV-C was examined. After irradiation, the cells were incubated for 0 or 30 minutes (control) or 0, 15, 30, and 45 minutes (ATF2) at 37°C and then collected. The electrophoretic mobility of ATF2 during

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SDS-PAGE was examined by protein immuno-blot analysis as described above. The two electrophoretic mobility forms of ATF2 were observed in ATF2-transfected cells, but not in control cells.

- 5 The phosphorylation state of wild-type (Thr^{69,71}) ATF2 and mutated (Ala^{69,71}) ATF2 was examined in cells labeled with [³²]P, treated without and with 40 J/m² UV-C, and then incubated at 37°C for 30 minutes (Hai et al. (1989) supra). The ATF2 proteins were isolated by
- 10 immunoprecipitation and analyzed by SDS-PAGE and autoradiography. The phosphorylated ATF2 proteins were examined by phosphoamino acid analysis as described above. Both forms of ATF2 contained phosphoserine, but only wild-type ATF2 contained phosphothreonine.
- 15 Tryptic phosphopeptide mapping was used to compare ATF2 phosphorylated *in vitro* by JNK1 with ATF2 phosphorylated in COS-1 cells. A map was also prepared with a sample composed of equal amounts of *in vivo* and *in vitro* phosphorylated ATF2 (Mix). Mutation of ATF2 at
- 20 Thr⁶⁹ and Thr⁷¹ resulted in the loss of two tryptic phosphopeptides in maps of ATF2 isolated from UV-irradiated cells. These phosphopeptides correspond to mono- and bis-phosphorylated peptides containing Thr⁶⁹ and Thr⁷¹. Both of these phosphopeptides were found in maps
- 25 of ATF2 phosphorylated by JNK1 *in vitro*.

Example 12. Inhibition of ATF2-Stimulated Gene Expression by Mutation of the Phosphorylation Sites Thr⁶⁹ and Thr⁷¹

- A fusion protein consisting of ATF2 and the GAL4
- 30 DNA binding domain was expressed in CHO cells as described above. The activity of the GAL4-ATF2 fusion protein was measured in co-transfection assays with the reporter plasmid pG5E1bLuc (Seth et al. (1992) J. Biol. Chem. 267:24796. The reporter plasmid contains five GAL4

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sites cloned upstream of a minimal promoter element and the firefly luciferase gene. Transfection efficiency was monitored with a control plasmid that expresses β -galactosidase (pCH110; Pharmacia-LKB Biotechnology). The luciferase and β -galactosidase activity detected in cell extracts was measured as the mean activity ratio of three experiments (Gupta et al. (1993) Proc. Natl. Acad. Sci. USA 90:3216). The results, shown in Table 1, demonstrate the importance of phosphorylation at Thr⁶⁹ and Thr⁷¹ for transcriptional activity.

TABLE 1. INHIBITION OF ATF-2 STIMULATED GENE EXPRESSION BY MUTATION OF THE PHOSPHORYLATION SITES THR^{69,71}

PROTEIN	LUCIFERASE ACTIVITY (Light Units/OD)
GAL4	45
GAL4-ATF2 (wild type)	320,000
GAL4-ATF2 (Ala ⁶⁹)	24,000
GAL4-ATF2 (Ala ⁷¹)	22,000
GAL4-ATF2 (Ala ^{69,71})	29,000
GAL4-ATF2 (Glu ⁶⁹)	27,000

Example 13. Effect of Dominant-Negative JNK1 Mutant on ATF2 Function

The luciferase reporter plasmid system was used to determine the effect of point mutations at the ATF2 phosphorylation sites Thr⁶⁹ and Thr⁷¹ in serum-treated CHO cells transfected with wild-type (Thr¹⁸³, Tyr¹⁸⁵) or mutant (Ala¹⁸³, Phe¹⁸⁵) JNK1. Control experiments were done with mock-transfected cells. The CHO cells were serum-starved for 18 hours and then incubated without or with serum for 4 hours. Expression of wild-type ATF2 caused a small increase in serum-stimulated ATF2 transcriptional activity. In contrast, mutant JNK1 inhibited both control and serum-stimulated ATF2 activity.

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Exempl 14. Effect of Tumor Suppressor Gene Product Rb
and Adenovirus Oncoprotein E1A on ATF2-
Stimulated Gene Expression

The effect of expression of the Rb tumor
5 suppressor gene product and adenovirus oncoprotein E1A on
ATF2 transcriptional activity were investigated with a
luciferase reporter plasmid and GAL4-ATF2 (residues 1-
505), as described above. Cells were transfected with
wild-type (Thr^{69,71}) or mutated (Ala^{69,71}) ATF2. No effect
10 of Rb or E1A on luciferase activity was detected in the
absence of GAL4-ATF2. Rb and E1A were found to increase
ATF2-stimulated gene expression of both wild-type and
mutated ATF2. However, mutated ATF2 caused a lower level
of reporter gene expression than did wild-type ATF2.
15 These results indicate a requirement for ATF2
phosphorylation (on Thr⁶⁹ and Thr⁷¹) plus either Rb or E1A
for maximal transcriptional activity.

Example 15. Substrate Specificity of p38 MAP Kinase

Substrate phosphorylation by p38 MAP kinase was
20 examined by incubation of bacterially-expressed p38 MAP
kinase with IκB, cMyc, EGF-R, cytoplasmic phospholipase
A₂ (cPLA₂), c-Jun, and mutated ATF2 (Thr^{69,71}) and ATP[γ-
³²P] (Rangneaud et al. (1995) J. Biol. Chem 270:7420.
GST-IκB was provided by Dr D. Baltimore (Massachusetts
25 Institute of Technology). GST-cMyc (Alvarez et al.
(1991) J. Biol. Chem. 266:15277), GST-EGF-R (residues
647-688) (Koland et al. (1990) Biochem. Biophys. Res.
Commun. 166:90), and GST-c-Jun (Dérillard et al. (1994)
supra) have been described. The phosphorylation reaction
30 was terminated after 30 minutes by addition of Laemmli
sample buffer. The phosphorylated proteins were resolved
by SDS-PAGE and detected by autoradiography. The rate
phosphorylation of the substrate proteins was quantitated
by PhosphorImager (Molecular Dynamics Inc.) analysis.

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The relative phosphorylation of ATF2, MBP, EGF-R, and I κ B was 1.0, 0.23, 0.04, and 0.001, respectively.

Example 16. Binding of p38 MAP Kinase to ATF2

Cell extracts expressing epitope-tagged JNK1 and
5 p38 MAP kinase were incubated with a GST fusion protein
containing the activation domain of ATF2 (residues 1-109)
immobilized on GSH agarose. The supernatant was removed
and the agarose was washed extensively. Western blot
analysis of the supernatant and agarose-bound fractions
10 was conducted as follows: proteins were fractionated by
SDS-PAGE, electrophoretically transferred to an
Immobilon-P membrane, and probed with monoclonal
antibodies to phosphotyrosine (PY20) and the Flag epitope
(M2). Immunocomplexes were detected using enhanced
15 chemiluminescence (Amersham International PLC). Control
experiments were performed using immobilized GST.

**Example 17. p38 MAP Kinase and JNK1 Activation by Pro-
Inflammatory Cytokines and Environmental
Stress**

20 The effect of phorbol ester, EGF, UV radiation,
osmotic stress, IL-1, tumor necrosis factor (TNF), and
LPS on p38 MAP kinase and JNK1 activity were measured in
immunocomplex protein kinase assays using ATP[γ -³²P] and
ATF2 as substrates. TNF α and IL-1 α were from Genzyme
25 Corp. Lipopolysaccharide (LPS) was isolated from
lyophilized *Salmonella minnesota* Re595 bacteria as
described (Mathison et al. (1988) J. Clin. Invest.
81:1925). Phorbol myristate acetate was from Sigma. EGF
was purified from mouse salivary glands (Davis (1988) J.
30 Biol. Chem. 263:9462). Kinase assays were performed
using immunoprecipitates of p38 and JNK. The
immunocomplexes were washed twice with kinase buffer
(described above), and the assays initiated by the

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addition of 1 μ g of ATF2 and 50 μ M [γ - 32 P]ATP (10 Ci/mmol) in a final volume of 25 μ l. The reactions were terminated after 30 minutes at 30°C by addition of Laemmli sample buffer. The phosphorylation of ATF2 was examined after SDS-PAGE by autoradiography, and the rate of ATF2 phosphorylation quantitated by PhosphorImager analysis.

The results are shown in Table 2. Exposure of HeLa cells to 10 nM phorbol myristate acetate very weakly activated p38 and JNK1. Similarly, treatment with 10 nM EGF only weakly activated p38 and JNK1. By contrast, treatment with 40 J/m² UV-C, 300 mM sorbitol, 10 ng/ml interleukin-1, and 10 ng/ml TNF α strongly activated p38 and JNK1 activity. The effect of LPS on the activity of p38 was examined using CHO cells that express human CD14. Exposure of CHO cells to 10 ng/ml LPS only slightly activated p38 and JNK1 activity.

TABLE 2. p38 AND JNK1 ACTIVATION BY PRO-INFLAMMATORY CYTOKINES AND ENVIRONMENTAL STRESS.

	Relative Protein Kinase Activity	
	JNK	p38
Control	1.0	1.0
Epidermal Growth Factor (10 nM)	1.9	2.1
Phorbol Ester (10 nM)	2.3	2.9
Lipopolysaccharide (10 ng/ml)	3.6	3.7
Osmotic Shock (300 mM sorbitol)	18.1	4.2
Tumor Necrosis Factor (10 ng/ml)	19.3	10.3
Interleukin-1 (10 ng/ml)	8.9	6.2
UV (40 J/m ²)	7.4	17.1

Example 18. p38 MAP Kinase Activation by Dual Phosphorylation on Tyr and Thr

COS-1 cells expressing wild-type (Thr¹⁸⁰, Tyr¹⁸²) or mutated (Ala¹⁸⁰, Phe¹⁸²) p38 MAP kinase were treated

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without and with UV-C (40 J/m^2). The cells were harvested 30 minutes following exposure with or without UV radiation. Control experiments were performed using mock-transfected cells. The level of expression of epitope-tagged p38 MAP kinase and the state of Tyr phosphorylation of p38 MAP kinase was examined by Western blot analysis using the M2 monoclonal antibody and the phosphotyrosine monoclonal antibody PY20. Immune complexes were detected by enhanced chemiluminescence.

10 Wild-type and mutant p38 were expressed at similar levels. Western blot analysis showed that UV radiation caused an increase in the Tyr phosphorylation of p38. The increased Tyr phosphorylation was confirmed by phosphoamino acid analysis of p38 isolated from

15 [^{32}P]phosphate-labeled cells. The results also showed that UV radiation increased Thr phosphorylation of p38. The increased phosphorylation on Tyr and Thr was blocked by mutated p38. Wild-type and mutated p38 were isolated from the COS-1 cells by immunoprecipitation. Protein

20 kinase activity was measured in the immune complex using [$\gamma\text{-}^{32}\text{P}$]ATP and GST-ATF2 as substrates. The phosphorylated GST-ATF2 was detected after SDS-PAGE by autoradiography. UV radiation resulted in a marked increase in the activity of wild-type p38, while the mutant p38 was found

25 to be catalytically inactive. These results show that p38 is activated by dual phosphorylation within the Thr-Gly-Tyr motif.

Example 19. MAP Kinase Phosphatase Inhibits p38 MAP Kinase Activation

30 The cells were treated without and with 40 J/m^2 UV-C. Control experiments were performed using mock-transfected cells (control) and cells transfected with the catalytically inactive mutated phosphatase mPAC1 (Cys²⁵⁷/Ser) and human MKP1. The activity of p38 MAP

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kinase was measured with an immunec mplex protein kinase assay employing [γ - 32 P]ATP and GST-ATF2 as substrates. The expression of PAC1 or MKP1 was found to inhibit p38 phosphorylation, demonstrating that p38 can be regulated
5 by the dual specificity phosphatases PAC1 and MKP1.

Example 20. Subcellular Distribution of p38 MAP Kinase

Epitope-tagged p38 MAP kinase was expressed in COS cells. The cells were treated without or with 40 J/m² UV radiation and then incubated for 60 minutes at
10 37°C. The p38 MAP kinase was detected by indirect immunofluorescence using the M2 monoclonal antibody. The images were acquired by digital imaging microscopy and processed for image restoration.

Immunocytochemistry. Coverslips (22mm x 22mm No.
15 1; Gold Seal Cover Glass; Becton-Dickinson) were pre-treated by boiling in 0.1 N HCl for 10 minutes, rinsed in distilled water, autoclaved and coated with 0.01% poly-L-lysine (Sigma; St. Louis MO). The coverslips were placed at the bottom of 35 mm multiwell tissue culture plates
20 (Becton Dickinson, UK). Transfected COS-1 cells were plated directly on the coverslips and allowed to adhere overnight in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum (Gibco-BRL). 24 hours post-transfection, the cells were rinsed once and
25 incubated at 37°C for 30 minutes in 25 mM Hepes, pH 7.4, 137 mM NaCl, 6 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 10 mM glucose. The cells were rinsed once with phosphate-buffered saline and the coverslips removed from the tissue culture wells. Cells were fixed in fresh 4%
30 paraformaldehyde in phosphate-buffered saline for 15 minutes at 22°C. The cells were permeabilized with 0.25% Triton X-100 in phosphate-buffered saline for 5 minutes and washed three times in DWB solution (150 mM NaCl, 15 mM Na citrate, pH 7.0, 2% horse serum, 1% (w/v) bovine

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serum albumin, 0.05% Triton X-100) for 5 minutes. The primary antibody (M2 anti-FLAG monoclonal antibody, Eastman-Kodak Co., New Haven, CT) was diluted 1:250 in DWB and applied to the cells in a humidified environment at 22°C for 1 hour. The cells were again washed three times as above and fluorescein isothiocyanate-conjugated goat anti-mouse Ig secondary antibody (Kirkegaard & Perry Laboratories Inc. Gaithersburg, MD) was applied at a 1:250 dilution for 1 hour at 22°C in a humidified environment. The cells were then washed three times in DWB and then mounted onto slides with Gel-Mount (Biomedica Corp. Foster City, CA) for immunofluorescence analysis. Control experiments were performed to assess the specificity of the observed immunofluorescence. No fluorescence was detected when the transfected cells were stained in the absence of the primary M2 monoclonal antibody, or mock-transfected cells.

Digital Imaging Microscopy and Image Restoration

Digital images of the fluorescence distribution in single cells were obtained using a Nikon 60x Planapo objective (numerical aperture = 1.4) on a Zeiss IM-35 microscope equipped for epifluorescence as previously described (Carrington et al. (1990) in: Non-invasive Techniques in Cell Biology (Fosbett & Grinstein, eds.), Wiley-Liss, NY; pp. 53-72; Fay et al. (1989) J. Microsci. 153:133-149). Images of various focal planes were obtained with a computer controlled focus mechanism and a thermoelectrically cooled charged-coupled device camera (model 220; Photometrics Ltd., Tucson, AZ). The exposure of the sample to the excitation source was determined by a computer-controlled shutter and wavelength selector system (MVI, Avon, MA). The charge-coupled device camera and microscope functions were controlled by a microcomputer, and the data acquired from the camera were transferred to a Silicon Graphics model 4D/GTX

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workstation (Mountainview, CA) for image processing. Images were corrected for non-uniformities in sensitivity and for the dark current of the charge coupled device detector. The calibration of the microscopy blurring was
5 determined by measuring the instrument's point spread function as a series of optical sections at $0.125\mu\text{m}$ intervals of a $0.3\mu\text{m}$ diameter fluorescently labeled latex bead (Molecular Probes Inc.). The image restoration algorithm used is based upon the theory of
10 ill-posed problems and obtains quantitative dye density values within the cell that are substantially more accurate than those in an un-processed image (Carrington et al. (1990) supra; Fay et al. (1989) supra). After image processing, individual optical sections of cells
15 were inspected and analyzed using computer graphics software on a Silicon Graphics workstation. p38 MAP kinase was observed at the cell surface, in the cytoplasm, and in the nucleus. After irradiation, an increased localization of cytoplasmic p38 to the
20 perinuclear region was detected.

Example 21. Activation of the MKK Signal Transduction Pathway by Osmotic Shock

CHO cells were co-transfected with the plasmid pCMV-Flag-Jnk1 and pRSV-Neo (Dérillard et al. (1994)
25 supra). A stable cell line expressing epitope-tagged Jnk1 (Flag; Immunex Corp.) was isolated by selection with Geneticin (Gibco-BRL). The cells were incubated with 0, 100, 150, 300, 600, or 1000 mM sorbitol for 1 hour at 37°C . The cells were collected in lysis buffer (20 mM
30 Tris, pH 7.4, 1% Triton X-100, 2 mM EDTA, 137 mM NaCl, 25 mM β -glycerophosphate, 1 mM orthovanadate, 2 mM pyrophosphate, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 $\mu\text{g/ml}$ leupeptin) and a soluble extract was obtained by centrifugation at 100,000 g for 30 minutes at

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4°C. The epitope-tagged JNK1 was isolated by immunoprecipitation with the monoclonal antibody M2 (Immunex Corp.). The immunoprecipitates were washed extensively with lysis buffer. Immune complex kinase assays were done in 25 µl of 25 mM Hepes, pH 7.4, 25 mM MgCl₂, 25 mM β-glycerophosphate, 2 mM dithiothreitol, 100 µM orthovanadate, and 50 µM ATP [γ-³²P] (10 Ci/mmol) with 2.5 µg of bacterially expressed c-Jun (residues 1-79) fused to glutathione-S-transferase (GST) as a substrate. The phosphorylation of c-Jun was examined after SDS-PAGE by autoradiography and PhosphorImager (Molecular Dynamics Inc.) analysis. JNK1 activation was observed at all concentrations of sorbitol exposure.

The time course of JNK1 protein kinase activation was measured in cells incubated in medium supplemented with 300 mM sorbitol as described above. Increased JNK1 activity was observed within 5 minutes of exposure to sorbitol, with maximum activity occurring after 15-30 minutes.

Mutation of JNK1 at the phosphorylation sites Thr¹⁸³ and Tyr¹⁸⁵ blocked the activation of JNK1 protein kinase activity by osmotic shock. CHO cells were transfected with vector, wild-type JNK1 (Thr¹⁸³, Tyr¹⁸⁵), and mutated JNK1 (Ala¹⁸³, Phe¹⁸⁵). The cells were incubated in medium supplemented without or with 300 mM sorbitol for 15 minutes before measurement of JNK1 protein kinase activity as described above. JNK1 activation was seen in the wild-type but not mutated JNK1.

30 Use

The MKK polypeptides and polynucleotides of the invention are useful for identifying reagents which modulate the MKK signal transduction pathways. Reagents that modulate an MKK signal transduction pathway can be

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identified by their effect on MKK synthesis, MKK phosphorylation, or MKK activity. For example, the effect of a reagent on MKK activity can be measured by the *in vitro* kinase assays described above. MKK is
5 incubated without (control) and with a test reagent under conditions sufficient to allow the components to react, then the effect of the test reagent on kinase activity is subsequently measured. Reagents that inhibit an MKK signal transduction pathway can be used in the treatment
10 of MKK-mediated disorders. Reagents that stimulate an MKK signal transduction pathway can be used in a number of ways, including induction of programmed cell death (apoptosis) in tissues. For example, the elimination of UV damaged cells can be used to prevent cancer.

15 Generally, for identification of a reagent that inhibits the MKK signal transduction pathway, the kinase assay is tested with a range of reagent concentrations, e.g., 1.0 nM to 100 mM, a MKK substrate, and a radioactive marker such as [γ -³²P]ATP. Appropriate
20 substrate molecules include p38, JNK1, JNK2, or ATF2. The incorporation of [³²]P into the substrate is determined, and the results obtained with the test reagent compared to control values. Of particular interest are reagents that result in inhibition of [³²]P
25 of about 80% or more.

Assays that test the effect of a reagent on MKK synthesis can also be used to identify compounds that inhibit MKK signal transduction pathways. The effect of the test reagent on MKK expression is measured by, for
30 example, Western blot analysis with an antibody specific for MKK. Antibody binding is visualized by autoradiography or chemiluminescence, and is quantitated. The effect of the test reagent on MKK mRNA expression can be examined, for example, by Northern blot analysis using
35 a polynucleotide probe or by polymerase chain reaction.

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Reagents found to inhibit MKK signal transduction pathways can be used as therapeutic agents for the treatment of MKK-mediated disorders. Such reagents are also useful in drug design for elucidation of the
5 specific molecular features needed to inhibit MKK signal transduction pathways.

In addition, the invention provides a method for the treatment of MKK-mediated stress-related and inflammatory disorders. The method includes
10 administration of an effective amount of a therapeutic reagent that inhibits MKK function. Suitable reagents inhibit either MKK activity or expression. The concentration of the reagent to be administered is determined based on a number of factors, including the
15 appropriate dosage, the route of administration, and the specific condition being treated. The appropriate dose of a reagent is determined by methods known to those skilled in the art including routine experimentation to optimize the dosage as necessary for the individual
20 patient and specific MKK-mediated disorder being treated. Specific therapeutically effective amounts appropriate for administration are readily determined by one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences. 18th ed., Gennaro, ed., Mack
25 Publishing Company, Easton, PA, 1990).

The invention provides methods for both acute and prophylactic treatment of stress-related and inflammatory disorders. For example, it is envisioned that ischemic heart disease will be treated during episodes of ischemia
30 and oxidative stress following reperfusion. In addition, a patient at risk for ischemia can be treated prior to ischemic episodes.

In another example, a therapeutic agent which inhibits MKK function or activity is administered to
35 control inflammatory responses by inhibiting the

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s or tion of inflammatory cytokines, including TNF and IL-1.

Stress-related proliferative disorders can also be treated by the method of the invention by administering a therapeutic reagent that inhibits MKK function or activity. Such therapeutic reagents can be used alone or in combination with other therapeutic reagents, for example, with chemotherapeutic agents in the treatment of malignancies. Indeed, the control of stress-activated MKK by the therapeutic reagents provided by this invention can modulate symptoms caused by other therapeutic strategies that induce stress.

The therapeutic reagents employed are compounds which inhibit MKK function or activity, including polynucleotides, polypeptides, and other molecules such as antisense oligonucleotides and ribozymes, which can be made according to the invention and techniques known to the art. Polyclonal or monoclonal antibodies (including fragments or derivatives thereof) that bind epitopes of MKK also can be employed as therapeutic reagents. Dominant-negative forms of MKK which effectively displace or compete with MKK for substrate binding and/or phosphorylation can be used to decrease protein kinase activity. Dominant-negative forms can be created by mutations within the catalytic domain of the protein kinases, as described above.

In some cases, augmentation of MKK activity is desirable, e.g., induction of apoptosis. The methods of the invention can be used to identify reagents capable of increasing MKK function or activity. Alternatively, increased activity is achieved by over-expression of MKK. When a MKK-mediated disorder is associated with underexpression of MKK, or expression of a mutant MKK polypeptide, a sense polynucleotide sequence (the DNA

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c ding strand) or MKK polypeptide can be introduced into the cell.

The antibodies of the invention can be administered parenterally by injection or by gradual
5 infusion over time. The monoclonal antibodies of the invention can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally.

Preparations for parenteral administration of a
10 polypeptide or an antibody of the invention include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as
15 ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed
20 oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose) and the like. Preservatives and other additives can also be present, such as, for example, antimicrobials, antioxidants, chelating agents,
25 and inert gases, and the like.

Polynucleotide sequences, including antisense sequences, can be therapeutically administered by various techniques known to those skilled in the art. Such therapy would achieve its therapeutic effect by
30 introduction of the MKK polynucleotide into cells of mammals having a MKK-mediated disorder. Delivery of MKK polynucleotides can be achieved using free polynucleotide or a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Especially

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preferred for therapeutic delivery of nucleotide sequences is the use of targeted liposomes.

Targeting of the therapeutic reagent to specific tissues is desirable to increase the efficiency of delivery. The targeting can be achieved by passive mechanisms via the route of administration. Active targeting to specific tissues can also be employed. The use of liposomes, colloidal suspensions, and viral vectors allows targeting to specific tissues by changing the composition of the formulation containing the therapeutic reagent, for example, by including molecules that act as receptors for components of the target tissues. Examples include sugars, glycolipids, polynucleotides, or proteins. These molecules can be included with the therapeutic reagent. Alternatively, these molecules can be included by indirect methods, for example, by inclusion of a polynucleotide that encodes the molecule, or by use of packaging systems that provide targeting molecules. Those skilled in the art will know, or will ascertain with the use of the teaching provided herein, which molecules and procedures will be useful for delivery of the therapeutic reagent to specific tissues.

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Davis, Roger J.
Raigneaud, Joel
Gupta, Shashi
Derijard, Benoit
- (ii) TITLE OF INVENTION: CYTOKINE-, STRESS-, AND
ONCOPROTEIN-ACTIVATED HUMAN PROTEIN KINASE
KINASES
- (iii) NUMBER OF SEQUENCES: 16
- (iv) CORRESPONDENCE ADDRESS:
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- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/530,950
 - (B) FILING DATE: 19-SEP-1995
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
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 - (B) REGISTRATION NUMBER: 32,983
 - (C) REFERENCE/DOCKET NUMBER: 07917/010001
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 - (C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2030 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- 50 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCTACGATCC TGGTGCAAGG COGGTGGATG CAGAGGCCAG TCCATATACC ACCCAGGCCT	120
GCGAGGAGCG TGGTCCCCAC CCATCCAGCC CATATGTGCA AGTGCCCTTG ACAGAGAGGC	180
TGGTCATATC CATGGTGACC ATTTATGGGC CACAACAGGT CCCCATCTGC GCAGTGAACC	240
CTGTGCTGAG CACCTTGAG ACGTGATCTT GCTTCGTCCT GCAGCACTGT GCGGGGCAGG	300
AAAATCCAAG AGGAAGAAGG ATCTACGGAT ATCCTGCATG TCCAAGCCAC CCGCACCCAA	360
CCCCACACCC CCCCAGAAC TGGACTCCCG GACCTTCATC ACCATTGGAG ACAGAACTT	420
TGAGGTGGAG GCTGATGACT TGGTGACCAT CTCAGAACTG GGCCGTGGAG CCTATGGGGT	480
GGTAGAGAAG GTGCGGCACG CCCAGAGCGG CACCATCATG GCCGTGAAGC GGATCCGGGC	540
CACCGTGAAC TCACAGGAGC AGAAGCGGCT GCTCATGGAC CTGGACATCA ACATGCGCAC	600
GGTCGACTGT TTCTACACTG TCACCTTCTA CGGGGCACTA TTCAGAGAGG GAGACGTGTG	660
GATCTGCATG GAGCTCATGG ACACATCCTT GGACAAGTTC TACCGGAAGG TGCTGGATAA	720
AAACATGACA ATTCCAGAGG ACATCCTTGG GGAGATTGCT GTGTCTATCG TGCGGGCCCT	780
GGAGCATCTG CACAGCAAGC TGTCGGTGAT CCACAGAGAT GTGAAGCCCT CCAATGTCCT	840
TATCAACAAG GAGGGCCATG TGAAGATGTG TGACTTTGGC ATCAGTGGCT ACTTGGTGGA	900
CTCTGTGGCC AAGACGATGG ATGCCGGCTG CAAGCCCTAC ATGGCCCTG AGAGGATCAA	960
CCCAGAGCTG AACCAGAAGG GCTACAATGT CAAGTCCGAC GTCTGGAGCC TGGGCATCAC	1020
CATGATTGAG ATGGCCATCC TGCGGTTCCC TTACGAGTCC TGGGGGACCC CGTTCCAGCA	1080
GCTGAAGCAG GTGGTGGAGG AGCCGTCCCC CCAGCTCCCA GCCGACCGTT TCTCCCCGA	1140
GTTTGTGGAC TTCCTGCTC AGTGCTGAG GAAGAACCCG GCAGAGCGTA TGAGCTACCT	1200
GGAGCTGATG GAGCACCCCT TCTTACCTT GCACAAAACC AAGAAGACGG ACATTGCTGC	1260
CTTCGTGAAG AAGATCCTGG GAGAAGACTC ATAGGGGCTG GGCCTCGGAC CCCACTCCGG	1320
CCCTCCAGAG CCCCACAGCC CCATCTGCGG GGCAGTGCT CACCCACACC ATAAGCTACT	1380
GCCATCCTGG CCCAGGGCAT CTGGGAGGAA CCGAGGGGGC TGCTCCACCC TGGCTCTGTG	1440
GCGAGCCATT TGTCCCAAGT GCCAAGAAG CAGACCATTG GGGCTCCCAG CCAGGCCCTT	1500
GTGGGCCCCA CCAGTGCCTC TCCCTGCTGC TCCTAGGACC CGTCTCCAGC TGCTGAGATC	1560
CTGGACTGAG GGGGCCTGGA TGCCCCCTGT GGATGCTGCT GCCCCTGCAC AGCAGGCTGC	1620
CAGTGCCTGG GTGGATGGGC CACCGCCTTG CCCAGCCTGG ATGCCATCCA AGTTGTATAT	1680
TTTTTTAATC TCTCGACTGA ATGGACTTTG CACACTTTGG CCCAGGGTGG CCACACCTCT	1740
ATCCCGGCTT TGGTGGGGG TACACAAGAG GGGATGAGTT GTGTGAATAC CCCAAGACTC	1800
CCATGAGGGA GATGCCATGA GCGCCCAAG GCCTTCCCTT GGCCTGGCA AACAGGGCCT	1860

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CTGCGGAGCA CACTGGCTCA CCCAGTCCTG CCGGCCACCG TTATCGGTGT CATTACACCTT 1920
 TCGTGTTTTT TTTAATTTAT CCTCTGTTGA TTTTCTCTTT TGCTTTATGG GTTTGGCTTG 1980
 TTTTCTTGC ATGTTTGGGA GCTGATCGCT TCTCCCCAC CCCCTAGGGG 2030

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 318 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Ser	Lys	Pro	Pro	Ala	Pro	Asn	Pro	Thr	Pro	Pro	Arg	Asn	Leu	Asp	1	5	10	15
Ser	Arg	Thr	Phe	Ile	Thr	Ile	Gly	Asp	Arg	Met	Phe	Glu	Val	Glu	Ala	20	25	30	
Asp	Asp	Leu	Val	Thr	Ile	Ser	Glu	Leu	Gly	Arg	Gly	Ala	Tyr	Gly	Val	35	40	45	
Val	Glu	Lys	Val	Arg	His	Ala	Gln	Ser	Gly	Thr	Ile	Met	Ala	Val	Lys	50	55	60	
Arg	Ile	Arg	Ala	Thr	Val	Asn	Ser	Gln	Glu	Gln	Lys	Arg	Leu	Leu	Met	65	70	75	80
Asp	Leu	Asp	Ile	Asn	Met	Arg	Thr	Val	Asp	Cys	Phe	Tyr	Thr	Val	Thr	85	90	95	
Phe	Tyr	Gly	Ala	Leu	Phe	Arg	Glu	Gly	Asp	Val	Trp	Ile	Cys	Met	Glu	100	105	110	
Leu	Met	Asp	Thr	Ser	Leu	Asp	Lys	Phe	Tyr	Arg	Lys	Val	Leu	Asp	Lys	115	120	125	
Asn	Met	Thr	Ile	Pro	Glu	Asp	Ile	Leu	Gly	Glu	Ile	Ala	Val	Ser	Ile	130	135	140	
Val	Arg	Ala	Leu	Glu	His	Leu	His	Ser	Lys	Leu	Ser	Val	Ile	His	Arg	145	150	155	160
Asp	Val	Lys	Pro	Ser	Asn	Val	Leu	Ile	Asn	Lys	Glu	Gly	His	Val	Lys	165	170	175	
Met	Cys	Asp	Phe	Gly	Ile	Ser	Gly	Tyr	Leu	Val	Asp	Ser	Val	Ala	Lys	180	185	190	
Thr	Met	Asp	Ala	Gly	Cys	Lys	Pro	Tyr	Met	Ala	Pro	Glu	Arg	Ile	Asn	195	200	205	
Pro	Glu	Leu	Asn	Gln	Lys	Gly	Tyr	Asn	Val	Lys	Ser	Asp	Val	Trp	Ser	210	215	220	
Leu	Gly	Ile	Thr	Met	Ile	Glu	Met	Ala	Ile	Leu	Arg	Phe	Pro	Tyr	Glu	225	230	235	240
Ser	Trp	Gly	Thr	Pro	Phe	Gln	Gln	Leu	Lys	Gln	Val	Val	Glu	Glu	Pro	245	250	255	

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Ser Pro Gln Leu Pro Ala Asp Arg Phe Ser Pro Glu Phe Val Asp Phe
 260 265 270

Thr Ala Gln Cys Leu Arg Lys Asn Pro Ala Glu Arg Met Ser Tyr Leu
 275 280 285

Glu Leu Met Glu His Pro Phe Phe Thr Leu His Lys Thr Lys Lys Thr
 290 295 300

Asp Ile Ala Ala Phe Val Lys Lys Ile Leu Gly Glu Asp Ser
 305 310 315

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1602 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TAGCTGCAGC ACAGCCTTCC CTAACGTTGC AACTGGGGGA AAAATCACTT TCCAGTCTGT	60
TTTGCAAGGT GTGCATTTCC ATCTTGATTG CCTGAAAGTC CATCTGCTGC ATCGGTCAAG	120
AGAAACTCCA CTTGCATGAA GATTGCACGC CTGCAGCTTG CATCTTTGTT GCAAACTAG	180
CTACAGAAGA GAAGCAAGGC AAAGTCTTTT GTGCTCCCCCT CCCCCATCAA AGGAAAGGGG	240
AAAATGTCTC AGTCGAAAGG CAAGAAGCGA AACCCTGGCC TTAAAATTCC AAAAGAAGCA	300
TTTGAACAAC CTCAGACCAG TTCCACACCA CCTAGAGATT TAGACTCCAA GGCTTGCAAT	360
TCTATTGGAA ATCAGAACTT TGAGGTGAAG GCAGATGACC TGGAGCCTAT AATGGAAGTG	420
GGACGAGGTG CGTACGGGGT GGTGGAGAAG ATGCGGCACG TGCCCAGCGG GCAGATCATG	480
GCAGTGAAGC GGATCCGAGC CACAGTAAAT AGCCAGGAAC AGAAACGGCT ACTGATGGAT	540
TTGGATATTT CCATGAGGAC GGTGGACTGT CCATTCACTG TCACCTTTTA TGGCGCACTG	600
TTTCGGGAGG GTGATGTGTG GATCTGCATG GAGCTCATGG ATACATCACT AGATAAATTC	660
TACAAACAAG TTATTGATAA AGGCCAGACA ATTCCAGAGG ACATCTTAGG GAAAATAGCA	720
GTTTCTATTG TAAAAGCATT AGAACATTTA CATAGTAAGC TGTCTGTCAT TCACAGAGAC	780
GTCAAGCCTT CTAATGTACT CATCAATGCT CTCGGTCAAG TGAAGATGTG CGATTTTGGA	840
ATCAGTGGCT ACTTGGTGGA CTCTGTTGCT AAAACAATTG ATGCAGGTG CAAACCATAC	900
ATGGCCCCTG AAAGAATAAA CCCAGAGCTC AACCAGAAGG GATACAGTGT GAAGTCTGAC	960
ATTTGGAGTC TGGGCATCAC GATGATTGAG TTGGCCATCC TTCGATTTCCT CTATGATTCA	1020
TGGGGAATC CATTTCAGCA GCTCAAACAG GTGGTAGAGG AGCCATCGCC ACAACTCCCA	1080
GCAGACAAGT TCTCTGCAGA GTTTGTTGAC TTTACCTCAC AGTGCTTAAA GAAGAATTCC	1140
AAAGAACGGC CTACATACCC AGAGCTAATG CAACATCCAT TTTTCACCCT ACATGAATCC	1200
AAAGGAACAG ATGTGGCATC TTTTGTAATA CTGATTCTTG GAGACTAAAA AGCAGTGGAC	1260

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TTAATCGGTT GACCCTACTG TGGATTGGTG GGTTCGGGG TGAAGCAAGT TCACTACAGC      1320
ATCAATAGAA AGTCATCTTT GAGATAATTT AACCTGCCT CTCAGAGGGT TTTCTCTCCC      1380
AATTTTCTTT TTAATCCCCC TCTTAAGGGG GCCTTGGAAT CTATAGTATA GAATGAACTG      1440
TCTAGATGGA TGAATTATGA TAAAGGCTTA GGACTTCAAA AGGTGATTAA ATATTTAATG      1500
ATGTGTCATA TGAGTCCTCA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA      1560
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AA                          1602

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 334 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Ser Gln Ser Lys Gly Lys Lys Arg Asn Pro Gly Leu Lys Ile Pro
1          5          10
Lys Glu Ala Phe Glu Gln Pro Gln Thr Ser Ser Thr Pro Pro Arg Asp
20        25        30
Leu Asp Ser Lys Ala Cys Ile Ser Ile Gly Asn Gln Asn Phe Glu Val
35        40        45
Lys Ala Asp Asp Leu Glu Pro Ile Met Glu Leu Gly Arg Gly Ala Tyr
50        55        60
Gly Val Val Glu Lys Met Arg His Val Pro Ser Gly Gln Ile Met Ala
65        70        75
Val Lys Arg Ile Arg Ala Thr Val Asn Ser Gln Glu Gln Lys Arg Leu
85        90        95
Leu Met Asp Leu Asp Ile Ser Met Arg Thr Val Asp Cys Pro Phe Thr
100       105       110
Val Thr Phe Tyr Gly Ala Leu Phe Arg Glu Gly Asp Val Trp Ile Cys
115      120      125
Met Glu Leu Met Asp Thr Ser Leu Asp Lys Phe Tyr Lys Gln Val Ile
130      135      140
Asp Lys Gly Gln Thr Ile Pro Glu Asp Ile Leu Gly Lys Ile Ala Val
145      150      155      160
Ser Ile Val Lys Ala Leu Glu His Leu His Ser Lys Leu Ser Val Ile
165      170      175
His Arg Asp Val Lys Pro Ser Asn Val Leu Ile Asn Ala Leu Gly Gln
180      185      190
Val Lys Met Cys Asp Phe Gly Ile Ser Gly Tyr Leu Val Asp Ser Val
195      200      205
Ala Lys Thr Ile Asp Ala Gly Cys Lys Pro Tyr Met Ala Pro Glu Arg
210      215      220

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Ile Asn Pro Glu Leu Asn Gln Lys Gly Tyr Ser Val Lys Ser Asp Ile
 225 230 235 240

Trp Ser Leu Gly Ile Thr Met Ile Glu Leu Ala Ile Leu Arg Phe Pro
 245 250 255

Tyr Asp Ser Trp Gly Thr Pro Phe Gln Gln Leu Lys Gln Val Val Glu
 260 265 270

Glu Pro Ser Pro Gln Leu Pro Ala Asp Lys Phe Ser Ala Glu Phe Val
 275 280 285

Asp Phe Thr Ser Gln Cys Leu Lys Lys Asn Ser Lys Glu Arg Pro Thr
 290 295 300

Tyr Pro Glu Leu Met Gln His Pro Phe Phe Thr Leu His Glu Ser Lys
 305 310 315 320

Gly Thr Asp Val Ala Ser Phe Val Lys Leu Ile Leu Gly Asp
 325 330

(2) INFORMATION FOR SEQ ID NO:5:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3497 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTAGGGTCCC CGGCGCCAGG CCACCCGGCC GTCAGCAGCA TGCAGGGTAA ACGCAAAGCA	60
CTGAAGTTGA ATTTTGCAA TCCACCTTTC AAATCTACAG CAAGGTTTAC TCTGAATCCC	120
AATCCTACAG GAGTTCAAAA CCCACACATA GAGAGACTGA GAACACACAG CATTGAGTCA	180
TCAGGAAAAC TGAAGATCTC CCCTGAACAA CACTGGGATT TCACTGCAGA GGACTTGAAA	240
GACCTTGGAG AAATTGGACG AGGAGCTTAT GGTTCGTCA ACAAATGGT CCACAAACCA	300
AGTGGGCAAA TAATGGCAGT TAAAGAATT CGGTCAACAG TGGATGAAA AGAACAAAA	360
CAACTTCTTA TGGATTGGA TGTAGTAATG CGGAGTAGTG ATTGCCATA CATTGTTCAG	420
TTTTATGGTG CACTCTTCAG AGAGGGTGAC TGTGGATCT GTATGGAAC CATGTCTACC	480
TCGTTTGATA AGTTTACAA ATATGTATAT AGTGTATTAG ATGATGTTAT TCCAGAAGAA	540
ATTTTAGGCA AAATCACTTT AGCAACTGTG AAAGCACTAA ACCACTTAA AGAAACTTG	600
AAAATTATTC ACAGAGATAT CAAACCTTCC AATATTCTTC TGGACAGAAG TGGAAATATT	660
AAGCTCTGTG ACTTCGGCAT CAGTGGACAG CTTGTGGACT CTATTGCCAA GACAAGAGAT	720
GCTGGCTGTA GGCCATACAT GGCACCTGAA AGAATAGACC CAAGCGCATC ACGACAAGGA	780
TATGATGTCC GCTCTGATGT CTGGAGTTG GGGATCACAT TGTATGAGTT GGCCACAGGC	840
CGATTTCCTT ATCCAAAGTG GAATAGTGT TTTGATCAAC TAACACAAGT CGTGAPAGGA	900
GATCCTCCGC AGCTGAGTAA TTCTGAGGAA AGGGAATTCT CCCCAGGTTT CATCAACTTT	960

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GTCAACTTGT GCCTTACGAA GGATGAATCC AAAAGGCCAA AGTATAAAGA GCTTCTGAAA	1020
CATCCCTTTA TTTTGATGTA TGAAGAACGT GCCGTTGAGG TCGCATGCTA TGTTTGTA	1080
ATCCTGGATC AAATGCCAGC TACTCCCAGC TCTCCCATGT ATGTCGATTG ATATCGTGCT	1140
ACATCAGACT CTAGAAAAAA GGGCTGAGAG GAAGCAAGAC GTAAAGAATT TTCATCCCCG	1200
ATCAGAGTGT TTTTATTGCT CGCCCAGACA CCATGTGCAA TAAGATTGGT GTTCGTTTCC	1260
ATCATGTCTG TATACTCCTG TCACCTAGAA CGTGCATCCT TGTAATACCT GATTGATCAC	1320
ACAGTGTTAG TGCTGGTCAG AGAGACCTCA TCCTGCTCTT TTGTGATGAA CATATTCATG	1380
AAATGTGGAA GTCAGTACGA TCAAGTTGTT GACTGTGATT AGATCACATC TTAAATTCAT	1440
TTCTAGACTC AAAACCTGGA GATGCAGCTA CTGGAATGGT GTTTTGTCAG ACTTCCAAAT	1500
CCTGGAAGGA CACAGTGATG AATGTACTAT ATCTGAACAT AGAAACTCGG GCTTGAGTGA	1560
GAAGAGCTTG CACAGCCAAC GAGACACATT GCCTTCTGGA GCTGGGAGAC AAAGGAGGAA	1620
TTTACTTTCT TCACCAAGTG CAATAGATTA CTGATGTGAT ATTCTGTTGC TTACAGTTA	1680
CAGTTGATGT TTGGGGATCG ATGTGCTCAG CCAAATTTCC TGTTTGAAAT ATCATGTTAA	1740
ATTAGAATGA ATTTATCTTT ACCAAAAACC ATGTTGCGTT CAAAGAGGTG AACATTAAAA	1800
TATAGAGACA GGACAGAATG TGTCTTTTC TCCTCTACCA GTCCTATTTT TCAATGGGAA	1860
GACTCAGGAG TCTGCCACTT GTCAAAGAAG GTGCTGATCC TAAGAATTTT TCATTCTCAG	1920
AATTCGGTGT GCTGCCAACT TGATGTTCCA CCTGCCACAA ACCACCAGGA CTGAAAGAAG	1980
AAAACAGTAC AGAAGGCAAA GTTTACAGAT GTTTTTAATT CTAGTATTTT ATCTGGAACA	2040
ACTTGTAGCA GCTATATATT TCCCCTTGGT CCCAAGCCTG ATACTTTAGC CATCATAACT	2100
CACTAACAGG GAGAAGTAGC TAGTAGCAAT GTGCCCTGAT TGATTAGATA AAGATTTCTA	2160
GTAGGCAGCA AAAGACCAAA TCTCAGTTGT TTGCTTCTTG CCATCACTGG TCCAGGTCTT	2220
CAGTTTCCGA ATCTCTTTCC CTTCCCCTGT GGTCTATTGT CGCTATGTGA CTTGCGCTTA	2280
ATCCAATATT TTGCCTTTTT TCTATATCAA AAAACCTTTA CAGTTAGCAG GGATGTTCTT	2340
TACCGAGGAT TTTTAACCCC CAATCTCTCA TAATCGCTAG TGTTTAAAG GCTAAGAATA	2400
GTGGGGCCCA ACCGATGTGG TAGGTGATAA AGAGGCATCT TTTCTAGAGA CACATTGGAC	2460
CAGATGAGGA TCCGAAACGG CAGCCTTTAC GTTCATCACC TGCTAGAACC TCTCGTAGTC	2520
CATCACCATT TCTTGGCATT GGAATTCTAC TGGAAAAAA TACAAAAGC AAAACAAAAC	2580
CCTCAGCACT GTTACAAGAG GCCATTTAAG TATCTTGTGC TTCTTCACTT ACCCATTAGC	2640
CAGGTTCTCA TTAGGTTTTG CTTGGGCCTC CCTGGCACTG AACCTTAGGC TTTGTATGAC	2700
AGTGAAGCAG CACTGTGAGT GGTCAAGCA CACTGGAATA TAAACAGTC ATGGCCTGAG	2760
ATGCAGGTGA TGCCATTACA GAACCAATC GTGGCACGTA TTGCTGTGTC TCCTCTCAGA	2820
GTGACAGTCA TAAATACTGT CAAACAATAA AGGGAGAATG GTGCTGTTTA AAGTCACATC	2880

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CCTGTAAATT GCAGAATTCA AAAGTGATTA TCTCTTTGAT CTACTTGCCT CATTTCCCTA      2940
TCTTCTCCCC CACGGTATCC TAAACTTTAG ACTTCCCACT GTTCTGAAAG GAGACATTGC      3000
TCTATGTCTG CCTTCGACCA CAGCAAGCCA TCATCCTCCA TTGCTCCCGG GGAACAAGA      3060
GGAATCTGTT TCTCTGCTGT CAACTTCCCA TCTGGCTCAG CATAGGGTCA CTTTGCCATT      3120
ATGCAAATGG AGATAAAGC AATTCTGGCT GTCCAGGAGC TAATCTGACC GTTCTATTGT      3180
GTGGATGACC ACATAAGAAG GCAATTTTAG TGTATTAATC ATAGATTATT ATAAACTATA      3240
AACTTAAGGG CAAGGAGTTT ATTACAATGT ATCTTTATTA AAACAAAAGG GTGTATAGTG      3300
TTCACAAACT GTGAAAATAG TGTAAAGAACT GTACATTGTG AGCTCTGTTT ATTTTCTCT      3360
TGTACCATAG AAAAATGTAT AAAAATTATC AAAAAGCTAA TGTGCAGGGA TATTGCCTTA      3420
TTTGTCTGTA AAAAATGGAG CTCAGTAACA TAACTGCTTC TTGGAGCTTT GGAATATTTT      3480
ATCCTGTATT CTGTGTTT                                     3497

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 363 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Gln Gly Lys Arg Lys Ala Leu Lys Leu Asn Phe Ala Asn Pro Pro
1           5           10           15
Phe Lys Ser Thr Ala Arg Phe Thr Leu Asn Pro Asn Pro Thr Gly Val
20           25           30
Gln Asn Pro His Ile Glu Arg Leu Arg Thr His Ser Ile Glu Ser Ser
35           40           45
Gly Lys Leu Lys Ile Ser Pro Glu Gln His Trp Asp Phe Thr Ala Glu
50           55           60
Asp Leu Lys Asp Leu Gly Glu Ile Gly Arg Gly Ala Tyr Gly Ser Val
65           70           75           80
Asn Lys Met Val His Lys Pro Ser Gly Gln Ile Met Ala Val Lys Arg
85           90           95
Ile Arg Ser Thr Val Asp Glu Lys Glu Gln Lys Gln Leu Leu Met Asp
100          105          110
Leu Asp Val Val Met Arg Ser Ser Asp Cys Pro Tyr Ile Val Gln Phe
115          120          125
Tyr Gly Ala Leu Phe Arg Glu Gly Asp Cys Trp Ile Cys Met Glu Leu
130          135          140
Met Ser Thr Ser Phe Asp Lys Phe Tyr Lys Tyr Val Tyr Ser Val Leu
145          150          155          160

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Asp Asp Val Ile Pro Glu Glu Ile Leu Gly Lys Ile Thr Leu Ala Thr
 165 170 175
 Val Lys Ala Leu Asn His Leu Lys Glu Asn Leu Lys Ile Ile His Arg
 180 185 190
 Asp Ile Lys Pro Ser Asn Ile Leu Leu Asp Arg Ser Gly Asn Ile Lys
 195 200 205
 Leu Cys Asp Phe Gly Ile Ser Gly Gln Leu Val Asp Ser Ile Ala Lys
 210 215 220
 Thr Arg Asp Ala Gly Cys Arg Pro Tyr Met Ala Pro Glu Arg Ile Asp
 225 230 235 240
 Pro Ser Ala Ser Arg Gln Gly Tyr Asp Val Arg Ser Asp Val Trp Ser
 245 250 255
 Leu Gly Ile Thr Leu Tyr Glu Leu Ala Thr Gly Arg Phe Pro Tyr Pro
 260 265 270
 Lys Trp Asn Ser Val Phe Asp Gln Leu Thr Gln Val Val Lys Gly Asp
 275 280 285
 Pro Pro Gln Leu Ser Asn Ser Glu Glu Arg Glu Phe Ser Pro Ser Phe
 290 295 300
 Ile Asn Phe Val Asn Leu Cys Leu Thr Lys Asp Glu Ser Lys Arg Pro
 305 310 315 320
 Lys Tyr Lys Glu Leu Leu Lys His Pro Phe Ile Leu Met Tyr Glu Glu
 325 330 335
 Arg Ala Val Glu Val Ala Cys Tyr Val Cys Lys Ile Leu Asp Gln Met
 340 345 350
 Pro Ala Thr Pro Ser Ser Pro Met Tyr Val Asp
 355 360

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3553 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAACAATGGC GGCTCCGAGC CCGAGCGGTG GCGGCGGCAG CGGCACCCCC GGCCCCGTAG	60
GGTCCCCGGC GCCAGGCCAC CCGGCCGTCA GCAGCATGCA GGGTAAACGC AAAGCACTGA	120
AGTTGAATTT TGCAAATCCA CCTTTCAAAT CTACAGCAAG GTTTACTCTG AATCCCAATC	180
CTACAGGAGT TCAAAACCCA CACATAGAGA GACTGAGAAC ACACAGCATT GAGTCATCAG	240
GAAACTGAA GATCTCCCT GAACAACACT GGGATTTAC TGCAGAGGAC TTGAAAGACC	300
TTGAGAAAT TGGACGAGGA GCTTAIGTT CTGTCAACAA AATGGTCCAC AAACCAAGTG	360
GGCAAATAAT GGCAGTTAA AGAATTCGGT CAACAGTGA TGAAAAAGAA CAAAAACAAC	420

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TTCTTATGGA	TITGGATGTA	GTAATGCCGA	GTAGTGATTG	CCCATACATT	GTTCAGTTTT	480
ATGGTGCACT	CTTCAGAGAG	GGTGACTGTT	GGATCTGTAT	GGAAGTCATG	TCTACCTCGT	540
TTGATAAGTT	TTACAAATAT	GTATATAGTG	TATTAGATGA	TGTTATTCCA	GAAGAAATTT	600
TAGGCAAAAT	CACTTTAGCA	ACTGTGAAAG	CACTAAACCA	CTTAAAGAA	AACTTGAAAA	660
TTATTACAG	AGATATCAAA	CCTTCCAATA	TTCTTCTGGA	CAGAAGTGGA	AATATTAAGC	720
TCTGTGACTT	CGGCATCAGT	GGACAGCTTG	TGGACTCTAT	TGCCAAGACA	AGAGATGCTG	780
GCTGTAGGCC	ATACATGGCA	CCTGAAAGAA	TAGACCCAAG	CGCATCACGA	CAAGGATATG	840
ATGTCGGCTC	TGATGTCTGG	AGTTTGGGGA	TCACATTGTA	TGAGTTGGCC	ACAGGCCGAT	900
TTCCTTATCC	AAAGTGGAAT	AGTGTATTTG	ATCAACTAAC	ACAAGTCGTG	AAAGGAGATC	960
CTCCGCAGCT	GAGTAATTCT	GAGGAAAGGG	AATTCTCCCC	GAGTTTCATC	AACTTTGTCA	1020
ACTTGTGCCT	TACGAAGGAT	GAATCCAAAA	GGCCAAAGTA	TAAAGAGCTT	CTGAAACATC	1080
CCTTTATTTT	GATGTATGAA	GAACGTGCCG	TTGAGGTGCG	ATGCTATGTT	TGTAAATCC	1140
TGGATCAAAT	GCCAGCTACT	CCCAGCTCTC	CCATGTATGT	CGATTGATAT	CGTGCTACAT	1200
CAGACTCTAG	AAAAAAGGGC	TGAGAGGAAG	CAAGACGTAA	AGAATTTTCA	TCCCGTATCA	1260
CAGTGTTTTT	ATTGCTCGCC	CAGACACCAT	GTGCAATAAG	ATTGGTGTTT	GTTTCCATCA	1320
TGTCTGTATA	CTCCTGTCAC	CTAGAACGTG	CATCCTTGTA	ATACCTGATT	GATCACACAG	1380
TGTTAGTGCT	GGTCAGAGAG	ACCTCATCCT	GCTCTTTTGT	GATGAACATA	TTCATGAAAT	1440
GTGGAAGTCA	GTACGATCAA	GTTGTTGACT	GTGATTAGAT	CACATCTTAA	ATTCATTTCT	1500
AGACTCAAAA	CCTGGAGATG	CAGCTACTGG	AATGGTGTTT	TGTCAGACTT	CCAAATCCTG	1560
GAAGGACACA	GTGATGAATG	TACTATATCT	GAACATAGAA	ACTCGGGCTT	GAGTGAGAAG	1620
AGCTTGACACA	GCCAACGAGA	CACATTGCCT	TCTGGAGCTG	GGAGACAAAG	GAGGAATTTA	1680
CTTTCTTCAC	CAAGTGCAAT	AGATTACTGA	TGTGATATTC	TGTTGCTTTA	CAGTTACAGT	1740
TGATGTTTGG	GGATCGATGT	GCTCAGCCAA	ATTTCCTGTT	TGAAATATCA	TGTTAAATTA	1800
GAATGAATTT	ATCTTTACCA	AAAACCATGT	TGCGTTCAAA	GAGGTGAACA	TTAAATATA	1860
GAGACAGGAC	AGAATGTGTT	CTTTTCTCCT	CTACCAGTCC	TATTTTTCAT	TGGGAAGACT	1920
CAGGAGTCTG	CCACTTGTC	AAGAAGGTGC	TGATCCTAAG	AATTTTTCAT	TCTCAGAATT	1980
CGGTGTGCTG	CCAAGTTGAT	GTTCCACCTG	CCACAAACCA	CCAGGACTGA	AAGAAGAAAA	2040
CAGTACAGAA	GGCAAAGTTT	ACAGATGTTT	TTAATTCTAG	TATTTTATCT	GGAACAACCTT	2100
GTAGCAGCTA	TATATTTCCC	CTTGGTCCCA	AGCCTGATAC	TTAGCCATC	ATAACTCACT	2160
AACAGGGAGA	AGTAGCTAGT	AGCAATGTGC	CTTGATTGAT	TAGATAAAGA	TTTCTAGTAG	2220
GCAGCAAAAG	ACCAAATCTC	AGTTGTTTGC	TTCTTGCCAT	CACTGGTCCA	GGTCTTCAGT	2280
TTCCGAATCT	CTTTCCCTTC	CCCTGTGGTC	TATTGTGCTG	ATGTGACTTG	CGCTTAATCC	2340

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AATATTTTGC CTTTTTCTA TATCAAAAA CCTTTACAGT TAGCAGGGAT GTTCCTTACC 2400
 GAGGATTTTT AACCCCCAAT CTCTCATAAT CGCTAGTGTT TAAAAGGCTA AGAATAGTGG 2460
 GGCCCAACCG ATGTGGTAGG TGATAAAGAG GCATCTTTTC TAGAGACACA TTGGACCAGA 2520
 TGAGGATCCG AAACGGCAGC CTTTACGTTC ATCACCTGCT AGAACCTCTC GTAGTCCATC 2580
 ACCATTTCTT GGCATTGGAA TTCTACTGGA AAAAAATACA AAAAGCAAAA CAAAACCCTC 2640
 AGCACTGTTA CAAGAGGCCA TTTAAGTATC TTGTGCTTCT TCACCTACCC ATTAGCCAGG 2700
 TTCTCATTAG GTTTTGCTTG GGCCTCCCTG GCACTGAACC TTAGGCTTTG TATGACAGTG 2760
 AAGCAGCACT GTGAGTGGTT CAAGCACACT GGAATATAAA ACAGTCATGG CCTGAGATGC 2820
 AGGTGATGCC ATTACAGAAC CAAATCGTGG CACGTATTGC TGTGTCTCCT CTCAGAGTGA 2880
 CAGTCATAAA TACTGTCAAA CAATAAAGGG AGAATGGTGC TGTTTAAAGT CACATCCCTG 2940
 TAAATTGCAG AATTCAAAAG TGATTATCTC TTTGATCTAC TTGCCTCATT TCCCTATCTT 3000
 CTCCCCCAG GTATCCTAAA CTTTAGACTT CCCACTGTTC TGAAAGGAGA CATTGCTCTA 3060
 TGTCTGCCTT CGACCACAGC AAGCCATCAT CCTCCATTGC TCCCGGGGAC TCAAGAGGAA 3120
 TCTGTTTCTC TGCTGTCAAC TTCCCATCTG GCTCAGCATA GGGTCACTTT GCCATTATGC 3180
 AAATGGAGAT AAAAGCAATT CTGGCTGTCC AGGAGCTAAT CTGACCGTTC TATTGTGTGG 3240
 ATGACCACAT AAGAAGGCAA TTTTAGTGTA TTAATCATAG ATTATTATAA ACTATAAACT 3300
 TAAGGGCAAG GAGTTTATTA CAATGTATCT TTATTAAAC AAAAGGGTGT ATAGTGTTCA 3360
 CAACTGTGA AAATAGTGTA AGAACTGTAC ATTGTGAGCT CTGGTTATTT TTCTCTTGTA 3420
 CCATAGAAAA ATGTATAAAA ATTATCAAAA AGCTAATGTG CAGGGATATT GCCTTATTTG 3480
 TCTGTAAAAA ATGGAGCTCA GTAACATAAC TGCTTCTTGG AGCTTTGGAA TATTTTATCC 3540
 TGTATTCTTG TTT 3553

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 393 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ala Ala Pro Ser Pro Ser Gly Gly Gly Gly Ser Gly Thr Pro Gly
 1 5 10 15
 Pro Val Gly Ser Pro Ala Pro Gly His Pro Ala Val Ser Ser Met Gln
 20 25 30
 Gly Lys Arg Lys Ala Leu Lys Leu Asn Phe Ala Asn Pro Pro Phe Lys
 35 40 45
 Ser Thr Ala Arg Phe Thr Leu Asn Pro Asn Pro Thr Gly Val Gln Asn
 50 55 60

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Pro His Ile Glu Arg Leu Arg Thr His Ser Ile Glu Ser Ser Gly Lys
 65 70 75 80
 Leu Lys Ile Ser Pro Glu Gln His Trp Asp Phe Thr Ala Glu Asp Leu
 85 90 95
 Lys Asp Leu Gly Glu Ile Gly Arg Gly Ala Tyr Gly Ser Val Asn Lys
 100 105 110
 Met Val His Lys Pro Ser Gly Gln Ile Met Ala Val Lys Arg Ile Arg
 115 120 125
 Ser Thr Val Asp Glu Lys Glu Gln Lys Gln Leu Leu Met Asp Leu Asp
 130 135 140
 Val Val Met Arg Ser Ser Asp Cys Pro Tyr Ile Val Gln Phe Tyr Gly
 145 150 155 160
 Ala Leu Phe Arg Glu Gly Asp Cys Trp Ile Cys Met Glu Leu Met Ser
 165 170 175
 Thr Ser Phe Asp Lys Phe Tyr Lys Tyr Val Tyr Ser Val Leu Asp Asp
 180 185 190
 Val Ile Pro Glu Glu Ile Leu Gly Lys Ile Thr Leu Ala Thr Val Lys
 195 200 205
 Ala Leu Met His Leu Lys Glu Asn Leu Lys Ile Ile His Arg Asp Ile
 210 215 220
 Lys Pro Ser Asn Ile Leu Leu Asp Arg Ser Gly Met Ile Lys Leu Cys
 225 230 235 240
 Asp Phe Gly Ile Ser Gly Gln Leu Val Asp Ser Ile Ala Lys Thr Arg
 245 250 255
 Asp Ala Gly Cys Arg Pro Tyr Met Ala Pro Glu Arg Ile Asp Phe Ser
 260 265 270
 Ala Ser Arg Gln Gly Tyr Asp Val Arg Ser Asp Val Trp Ser Leu Gly
 275 280 285
 Ile Thr Leu Tyr Glu Leu Ala Thr Gly Arg Phe Pro Tyr Pro Lys Trp
 290 295 300
 Asn Ser Val Phe Asp Gln Leu Thr Gln Val Val Lys Gly Asp Pro Pro
 305 310 315 320
 Gln Leu Ser Asn Ser Glu Glu Arg Glu Phe Ser Pro Ser Phe Ile Asn
 325 330 335
 Phe Val Asn Leu Cys Leu Thr Lys Asp Glu Ser Lys Arg Pro Lys Tyr
 340 345 350
 Lys Glu Leu Leu Lys His Pro Phe Ile Leu Met Tyr Glu Glu Arg Ala
 355 360 365
 Val Glu Val Ala Cys Tyr Val Cys Lys Ile Leu Asp Gln Met Pro Ala
 370 375 380
 Thr Pro Ser Ser Pro Met Tyr Val Asp
 385 390

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(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3576 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTCCCAACAA TGGCGGCTCC GAGCCCGAGC GGCGGCGGCG GCTCCGGGGG CGGCAGCGGC	60
AGCGGCACCC CCGGCCCCGT AGGGTCCCCG GCGCCAGGCC ACCCGGCCGT CAGCAGCATG	120
CAGGGTAAAC GCAAAGCACT GAAGTTGAAT TTTGCAAATC CACCTTTCAA ATCTACAGCA	180
AGGTTTACTC TGAATCCCAA TCCTACAGGA GTTCAAAACC CACACATAGA GAGACTGAGA	240
ACACACAGCA TTGAGTCATC AGGAAACTG AAGATCTCCC CTGAACAACA CTGGGATTTC	300
ACTGCAGAGG ACTTGAAAGA CCTTGAGAA ATTGGACGAG GAGCTTATGG TTCTGTCAAC	360
AAAATGGTCC ACAAACCAAG TGGGCAAATA ATGGCAGTTA AAAGAATTCT GTCAACAGTG	420
GATGAAAAAG AACAAAAACA ACTTCTTATG GATTGGATG TAGTAATGCG GAGTAGTGAT	480
TGCCCATACA TTGTTCACTT TTATGGTGCA CTCTTCAGAG AGGGTGAAGT TTGGATCTGT	540
ATGGAAGTCA TGTCTACCTC GTTGATAAG TTTTACAAAT ATGTATATAG TGTATTAGAT	600
GATGTTATTC CAGAAGAAAT TTAGGCAAA ATCACTTTAG CAACTGTGAA AGCACTAAAC	660
CACTTAAAAG AAAACTTGAA AATTATTCAC AGAGATATCA AACCTTCAA TATTCTTCTG	720
GACAGAAGTG GAAATATTAA GCTCTGTGAC TTCGGCATCA GTGGACAGCT TGTGGACTCT	780
ATTGCCAAGA CAAGAGATGC TGGCTGTAGG CCATACATGG CACCTGAAAG AATAGACCCA	840
AGCGCATCAC GACAAGGATA TGATGTCCGC TCTGATGTCT GGAGTTTGGG GATCACATTG	900
TATGAGTTGG CCACAGGCCG ATTTCTTAT CCAAAGTGA ATAGTGTATT TGATCAACTA	960
ACACAAGTCG TGAAAGGAGA TCCTCCGCAG CTGAGTAATT CTGAGGAAAG GGAATTCTCC	1020
CCGAGTTTCA TCACTTTGT CAACTTGTGC CTTACGAAG ATGAATCCAA AAGGCCAAAG	1080
TATAAGAGC TTCTGAAACA TCCCTTTATT TTGATGTATG AAGAACGTGC CGTTGAGGTC	1140
GCATGCTATG TTTGTAAAAT CCTGGATCAA ATGCCAGCTA CTCCAGCTC TCCCATGTAT	1200
GTCGATTGAT ATCGCTGCTA CATCAGACTC TAGAAAAAG GGCTGAGAGG AAGCAAGACG	1260
TAAAGAATTT TCATCCCGTA TCACAGTGTT TTTATTGCTC GCCCAGACAC CATGTGCAAT	1320
AAGATTGGTG TTCGTTTCCA TCATGTCTGT ATACTCTGT CACCTAGAAC GTGCATCCTT	1380
GTAATACCTG ATTGATCACA CAGTGTAGT GCTGGTCAGA GAGACCTCAT CCTGCTCTTT	1440
TGTGATGAAC ATATTCATGA AATGTGGAAG TCAGTACGAT CAAGTTGTTG ACTGTGATTA	1500
GATCACATCT TAAATTCATT TCTAGACTCA AAACCTGGAG ATGCAGCTAC TGGAAATGGTG	1560
TTTTGTCAGA CTTCCAAATC CTGGAAGGAC ACAGTGATGA ATGTACTATA TCTGAACATA	1620

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GAAACTCGGG	CTTGAGTGAG	AAGAGCTTGC	ACAGCCAACG	AGACACATTG	CCTTCTGGAG	1680
CTGGGAGACA	AAGGAGGAAT	TTACTTCTCT	CACCAAGTGC	AATAGATTAC	TGATGTGATA	1740
TTCTGTTGCT	TTACAGTTAC	AGTTGATGTT	TGGGGATCGA	TGTGCTCAGC	CAAATTTCTCT	1800
GTTTGAAATA	TCATGTTAAA	TTAGAATGAA	TTTATCTTTA	CCAAAAACCA	TGTTGCGTTC	1860
AAAGAGGTGA	ACATTAAAAAT	ATAGAGACAG	GACAGAATGT	GTTCTTTTCT	CCTCTACCAG	1920
TCCTATTTTT	CAATGGGAAG	ACTCAGGAGT	CTGCCACTTG	TCAAAGAAGG	TGCTGATCCT	1980
AAGAATTTTT	CATTCTCAGA	ATTCGGTGTG	CTGCCAAGTT	GATGTTCCAC	CTGCCACAAA	2040
CCACCAGGAC	TGAAAGAAGA	AAACAGTACA	GAAGGCRAAG	TTTACAGATG	TTTTTAATTC	2100
TAGTATTTTA	TCTGGAACAA	CTTGTAGCAG	CTATATATTT	CCCCTTGGTC	CCAAGCCTGA	2160
TACTTTAGCC	ATCATAACTC	ACTAACAGGG	AGAAGTAGCT	AGTAGCAATG	TGCCTTGATT	2220
GATTAGATAA	AGATTCTAG	TAGGCAGCAA	AAGACCAAAT	CTCAGTTGTT	TGCTTCTTGC	2280
CATCACTGGT	CCAGGTCTTC	AGTTTCCGAA	TCTCTTTCCC	TTCCCCTGTG	GTCTATTGTC	2340
GCTATGTGAC	TTGCGCTTAA	TCCAATATTT	TGCCTTTTTT	CTATATCAAA	AAACCTTTAC	2400
AGTTAGCAGG	GATGTTCCCT	ACCGAGGATT	TTTAACCCCC	AATCTCTCAT	AATCGCTAGT	2460
GTTTAAAGG	CTAAGAATAG	TGGGGCCCAA	COGATGTGGT	AGGTGATAAA	GAGGCATCTT	2520
TTCTAGAGAC	ACATTGGACC	AGATGAGGAT	CCGAAACGGC	AGCCTTTACG	TTCATCACCT	2580
GCTAGAACCT	CTCGTAGTCC	ATCACCATTT	CTTGGCATTG	GAATTCTACT	GGAAAAAAT	2640
ACAAAAAGCA	AAACAAAACC	CTCAGCACTG	TTACAAGAGG	CCATTTAAGT	ATCTTGCTGT	2700
TCTTCACTTA	CCCATTAGCC	AGGTTCTCAT	TAGGTTTTGC	TTGGGCCTCC	CTGGCACTGA	2760
ACCTTAGGCT	TTGTATGACA	GTGAAGCAGC	ACTGTGAGTG	GTTCAAGCAC	ACTGGAATAT	2820
AAAACAGTCA	TGGCCTGAGA	TGCAGGTGAT	GCCATTACAG	AACCAAATCG	TGGCACGTAT	2880
TGCTGTGTCT	CCTCTCAGAG	TGACAGTCAT	AAATACTGTC	AAACAATAAA	GGGAGAATGG	2940
TGCTGTTTAA	AGTCACATCC	CTGTAAATTG	CAGAATTCAA	AAGTGATTAT	CTCTTTGATC	3000
TACTTGCCCTC	ATTTCCCTAT	CTTCTCCCCC	ACGGTATCCT	AAACTTTAGA	CTTCCCCTG	3060
TTCTGAAAGG	AGACATTGCT	CTATGTCTGC	CTTCGACCAC	AGCAAGCCAT	CATCCTCCAT	3120
TGCTCCCGGG	GACTCAAGAG	GAATCTGTTT	CTCTGCTGTC	AACTTCCCAT	CTGGCTCAGC	3180
ATAGGGTCAC	TTTGCCATTA	TGCAAATGGA	GATAAAAGCA	ATTCTGGCTG	TCCAGGAGCT	3240
AATCTGACCG	TTCTATTGTG	TGGATGACCA	CATAAGAAGG	CAATTTTAGT	GTATTAATCA	3300
TAGATTATTA	TAAACTATAA	ACTTAAGGGC	AAGGAGTTTA	TTACAATGTA	TCTTTATTAA	3360
AACAAAAGGG	TGTATAGTGT	TCACAACTG	TGAAAATAGT	GTAAGAACTG	TACATTGTGA	3420
GCTCTGGTTA	TTTTTCTCTT	GTACCATAGA	AAAATGTATA	AAAATTATCA	AAAAGCTAAT	3480
GTGCAGGGAT	ATTGCCTTAT	TTGTCTGTAA	AAAATGGAGC	TCAGTAACAT	AACTGCTTCT	3540

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TGGAGCTTTG GAATATTTTA TCCTGTATTC TTGTTT

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(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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Met Ala Ala Pro Ser Pro Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser
1          5          10          15
Gly Ser Gly Thr Pro Gly Pro Val Gly Ser Pro Ala Pro Gly His Pro
20          25          30
Ala Val Ser Ser Met Gln Gly Lys Arg Lys Ala Leu Lys Leu Asn Phe
35          40          45
Ala Asn Pro Pro Phe Lys Ser Thr Ala Arg Phe Thr Leu Asn Pro Asn
50          55          60
Pro Thr Gly Val Gln Asn Pro His Ile Glu Arg Leu Arg Thr His Ser
65          70          75          80
Ile Glu Ser Ser Gly Lys Leu Lys Ile Ser Pro Glu Gln His Trp Asp
85          90          95
Phe Thr Ala Glu Asp Leu Lys Asp Leu Gly Glu Ile Gly Arg Gly Ala
100         105         110
Tyr Gly Ser Val Asn Lys Met Val His Lys Pro Ser Gly Gln Ile Met
115         120         125
Ala Val Lys Arg Ile Arg Ser Thr Val Asp Glu Lys Glu Gln Lys Gln
130         135         140
Leu Leu Met Asp Leu Asp Val Val Met Arg Ser Ser Asp Cys Pro Tyr
145         150         155         160
Ile Val Gln Phe Tyr Gly Ala Leu Phe Arg Glu Gly Asp Cys Trp Ile
165         170         175
Cys Met Glu Leu Met Ser Thr Ser Phe Asp Lys Phe Tyr Lys Tyr Val
180         185         190
Tyr Ser Val Leu Asp Asp Val Ile Pro Glu Glu Ile Leu Gly Lys Ile
195         200         205
Thr Leu Ala Thr Val Lys Ala Leu Asn His Leu Lys Glu Asn Leu Lys
210         215         220
Ile Ile His Arg Asp Ile Lys Pro Ser Asn Ile Leu Leu Asp Arg Ser
225         230         235         240
Gly Asn Ile Lys Leu Cys Asp Phe Gly Ile Ser Gly Gln Leu Val Asp
245         250         255
S r Ile Ala Lys Thr Arg Asp Ala Gly Cys Arg Pro Tyr M t Ala Pro
260         265         270

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Glu Arg Ile Asp Pro Ser Ala Ser Arg Gln Gly Tyr Asp Val Arg Ser
 275 280 285
 Asp Val Trp Ser Leu Gly Ile Thr Leu Tyr Glu Leu Ala Thr Gly Arg
 290 295 300
 Phe Pro Tyr Pro Lys Trp Asn Ser Val Phe Asp Gln Leu Thr Gln Val
 305 310 315 320
 Val Lys Gly Asp Pro Gln Leu Ser Asn Ser Glu Glu Arg Glu Phe
 325 330 335
 Ser Pro Ser Phe Ile Asn Phe Val Asn Leu Cys Leu Thr Lys Asp Glu
 340 345 350
 Ser Lys Arg Pro Lys Tyr Lys Glu Leu Leu Lys His Pro Phe Ile Leu
 355 360 365
 Met Tyr Glu Glu Arg Ala Val Glu Val Ala Cys Tyr Val Cys Lys Ile
 370 375 380
 Leu Asp Gln Met Pro Ala Thr Pro Ser Ser Pro Met Tyr Val Asp
 385 390 395

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 393 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Pro Lys Lys Lys Pro Thr Pro Ile Gln Leu Asn Pro Ala Pro Asp
 1 5 10 15
 Gly Ser Ala Val Asn Gly Thr Ser Ser Ala Glu Thr Asn Leu Glu Ala
 20 25 30
 Leu Gln Lys Lys Leu Glu Glu Leu Glu Leu Asp Glu Gln Gln Arg Lys
 35 40 45
 Arg Leu Glu Ala Phe Leu Thr Gln Lys Gln Lys Val Gly Glu Leu Lys
 50 55 60
 Asp Asp Asp Phe Glu Lys Ile Ser Glu Leu Gly Ala Gly Asn Gly Gly
 65 70 75 80
 Val Val Phe Lys Val Ser His Lys Pro Ser Gly Leu Val Met Ala Arg
 85 90 95
 Lys Leu Ile His Leu Glu Ile Lys Pro Ala Ile Arg Asn Gln Ile Ile
 100 105 110
 Arg Glu Leu Gln Val Leu His Glu Cys Asn Ser Pro Tyr Ile Val Gly
 115 120 125
 Phe Tyr Gly Ala Phe Tyr Ser Asp Gly Glu Ile Ser Ile Cys Met Glu
 130 135 140
 His Met Asp Gly Gly Ser Leu Asp Gln Val Leu Lys Lys Ala Gly Arg
 145 150 155 160

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Ile Pro Glu Gln Ile Leu Gly Lys Val Ser Ile Ala Val Ile Lys Gly
 165 170 175
 Leu Thr Tyr Leu Arg Glu Lys His Lys Ile Met His Arg Asp Val Lys
 180 185 190
 Pro Ser Asn Ile Leu Val Asn Ser Arg Gly Glu Ile Lys Leu Cys Asp
 195 200 205
 Phe Gly Val Ser Gly Gln Leu Ile Asp Ser Met Ala Asn Ser Phe Val
 210 215 220
 Gly Thr Arg Ser Tyr Met Ser Pro Glu Arg Leu Gln Gly Thr His Tyr
 225 230 235 240
 Ser Val Gln Ser Asp Ile Trp Ser Met Gly Leu Ser Leu Val Glu Met
 245 250 255
 Ala Val Gly Arg Tyr Pro Ile Pro Pro Pro Asp Ala Lys Glu Leu Glu
 260 265 270
 Leu Met Phe Gly Cys Gln Val Glu Gly Asp Ala Ala Glu Thr Pro Pro
 275 280 285
 Arg Pro Arg Thr Pro Gly Arg Pro Leu Ser Ser Tyr Gly Met Asp Ser
 290 295 300
 Arg Pro Pro Met Ala Ile Phe Glu Leu Leu Asp Tyr Ile Val Asn Glu
 305 310 315 320
 Pro Pro Pro Lys Leu Pro Ser Gly Val Phe Ser Leu Glu Phe Gln Asp
 325 330 335
 Phe Val Asn Lys Cys Leu Ile Lys Asn Pro Ala Glu Arg Ala Asp Leu
 340 345 350
 Lys Gln Leu Met Val His Ala Phe Ile Lys Arg Ser Asp Ala Glu Glu
 355 360 365
 Val Asp Phe Ala Gly Trp Leu Cys Ser Thr Ile Gly Leu Asn Gln Pro
 370 375 380
 Ser Thr Pro Thr His Ala Ala Gly Val
 385 390

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 400 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Leu Ala Arg Arg Lys Pro Val Leu Pro Ala Leu Thr Ile Asn Pro
 1 5 10 15
 Thr Ile Ala Glu Gly Pro Ser Pro Thr Ser Glu Gly Ala Ser Glu Ala
 20 25 30
 Asn Leu Val Asp Leu Gln Lys Lys Leu Glu Glu Leu Glu Leu Asp Glu
 35 40 45

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Gln Gln Lys Lys Arg Leu Glu Ala Phe Leu Thr Gln Lys Ala Lys Val
 50 55 60
 Ser Glu Leu Lys Asp Asp Asp Phe Glu Arg Ile Ser Glu Leu Gly Ala
 65 70 75 80
 Gly Asn Gly Gly Val Val Thr Lys Val Gln His Arg Pro Ser Gly Leu
 85 90 95
 Ile Met Ala Arg Lys Leu Ile His Leu Glu Ile Lys Pro Ala Ile Arg
 100 105 110
 Asn Gln Ile Ile Arg Glu Leu Gln Val Leu His Glu Cys Asn Ser Pro
 115 120 125
 Tyr Ile Val Gly Phe Tyr Gly Ala Phe Tyr Ser Asp Gly Glu Ile Ser
 130 135 140
 Ile Cys Met Glu His Met Asp Gly Gly Ser Leu Asp Gln Val Leu Lys
 145 150 155 160
 Glu Ala Lys Arg Ile Pro Glu Glu Ile Leu Gly Lys Val Ser Ile Ala
 165 170 175
 Val Leu Arg Gly Leu Ala Tyr Leu Arg Glu Lys His Gln Ile Met His
 180 185 190
 Arg Asp Val Lys Pro Ser Asn Ile Leu Val Asn Ser Arg Gly Glu Ile
 195 200 205
 Lys Leu Cys Asp Phe Gly Val Ser Gly Gln Leu Ile Asp Ser Met Ala
 210 215 220
 Asn Ser Phe Val Gly Thr Arg Ser Tyr Met Ala Pro Glu Arg Leu Gln
 225 230 235 240
 Gly Thr His Tyr Ser Val Gln Ser Asp Ile Trp Ser Met Gly Leu Ser
 245 250 255
 Leu Val Glu Leu Ala Val Gly Arg Tyr Pro Ile Pro Pro Pro Asp Ala
 260 265 270
 Lys Glu Leu Glu Ala Ile Phe Gly Arg Pro Val Val Asp Gly Glu Glu
 275 280 285
 Gly Glu Pro His Ser Ile Ser Pro Arg Pro Arg Pro Pro Gly Arg Pro
 290 295 300
 Val Ser Gly His Gly Met Asp Ser Arg Pro Ala Met Ala Ile Phe Glu
 305 310 315 320
 Leu Leu Asp Tyr Ile Val Asn Glu Pro Pro Pro Lys Leu Pro Asn Gly
 325 330 335
 Val Phe Thr Pro Asp Phe Gln Glu Phe Val Asn Lys Cys Leu Ile Lys
 340 345 350
 Asn Pro Ala Glu Arg Ala Asp Leu Lys Met Leu Thr Asn His Thr Phe
 355 360 365
 Ile Lys Arg Ser Glu Val Glu Glu Val Asp Phe Ala Gly Trp Leu Cys
 370 375 380

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Lys Thr Leu Arg Leu Asn Gln Pro Gly Thr Pro Thr Arg Thr Ala Val
 385 390 395 400

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 668 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Glu Asp Lys Phe Ala Asn Leu Ser Leu His Glu Lys Thr Gly Lys
 1 5 10 15

Ser Ser Ile Gln Leu Asn Glu Gln Thr Gly Ser Asp Asn Gly Ser Ala
 20 25 30

Val Lys Arg Thr Ser Ser Thr Ser Ser His Tyr Asn Asn Ile Asn Ala
 35 40 45

Asp Leu His Ala Arg Val Lys Ala Phe Gln Glu Gln Arg Ala Leu Lys
 50 55 60

Arg Ser Ala Ser Val Gly Ser Asn Gln Ser Glu Gln Asp Lys Gly Ser
 65 70 75 80

Ser Gln Ser Pro Lys His Ile Gln Gln Ile Val Asn Lys Pro Leu Pro
 85 90 95

Pro Leu Pro Val Ala Gly Ser Ser Lys Val Ser Gln Arg Met Ser Ser
 100 105 110

Gln Val Val Gln Ala Ser Ser Lys Ser Thr Leu Lys Asn Val Leu Asp
 115 120 125

Asn Gln Glu Thr Gln Asn Ile Thr Asp Val Asn Ile Asn Ile Asp Thr
 130 135 140

Thr Lys Ile Thr Ala Thr Thr Ile Gly Val Asn Ile Gly Leu Pro Ala
 145 150 155 160

Thr Asp Ile Thr Pro Ser Val Ser Asn Thr Ala Ser Ala Thr His Lys
 165 170 175

Ala Gln Leu Leu Asn Pro Asn Arg Arg Ala Pro Arg Arg Pro Leu Ser
 180 185 190

Thr Gln His Pro Thr Arg Pro Asn Val Ala Pro His Lys Ala Pro Ala
 195 200 205

Ile Ile Asn Thr Pro Lys Gln Ser Leu Ser Ala Arg Arg Gly Leu Lys
 210 215 220

Leu Pro Pro Gly Gly Met Ser Leu Lys Met Pro Thr Lys Thr Ala Gln
 225 230 235 240

Gln Pro Gln Gln Phe Ala Pro Ser Pro Ser Asn Lys Lys His Ile Glu
 245 250 255

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Thr Leu Ser Asn Ser Lys Val Val Glu Gly Lys Arg Ser Asn Pro Gly
 260 265 270
 Ser Leu Ile Asn Gly Val Gln Ser Thr Ser Thr Ser Ser Thr Glu
 275 280 285
 Gly Pro His Asp Thr Val Gly Thr Thr Pro Arg Thr Gly Asn Ser Asn
 290 295 300
 Asn Ser Ser Asn Ser Gly Ser Ser Gly Gly Gly Gly Leu Phe Ala Asn
 305 310 315 320
 Phe Ser Lys Tyr Val Asp Ile Lys Ser Gly Ser Leu Asn Phe Ala Gly
 325 330 335
 Lys Leu Ser Leu Ser Ser Lys Gly Ile Asp Phe Ser Asn Gly Ser Ser
 340 345 350
 Ser Arg Ile Thr Leu Asp Glu Leu Glu Phe Leu Asp Glu Leu Gly His
 355 360 365
 Gly Asn Tyr Gly Asn Val Ser Lys Val Leu His Lys Pro Thr Asn Val
 370 375 380
 Ile Met Ala Thr Lys Glu Val Arg Leu Glu Leu Asp Glu Ala Lys Phe
 385 390 395 400
 Arg Gln Ile Leu Met Glu Leu Glu Val Leu His Lys Cys Asn Ser Pro
 405 410 415
 Tyr Ile Val Asp Phe Tyr Gly Ala Phe Phe Ile Glu Gly Ala Val Tyr
 420 425 430
 Met Cys Met Glu Tyr Met Asp Gly Gly Ser Leu Asp Lys Ile Tyr Asp
 435 440 445
 Glu Ser Ser Glu Ile Gly Gly Ile Asp Glu Pro Gln Leu Ala Phe Ile
 450 455 460
 Ala Asn Ala Val Ile His Gly Leu Lys Glu Leu Lys Glu Gln His Asn
 465 470 475 480
 Ile Ile His Arg Asp Val Lys Pro Thr Asn Ile Leu Cys Ser Ala Asn
 485 490 495
 Gln Gly Thr Val Lys Leu Cys Asp Phe Gly Val Ser Gly Asn Leu Val
 500 505 510
 Ala Ser Leu Ala Lys Thr Asn Ile Gly Cys Gln Ser Tyr Met Ala Pro
 515 520 525
 Glu Arg Ile Lys Ser Leu Asn Pro Asp Arg Ala Thr Tyr Thr Val Gln
 530 535 540
 Ser Asp Ile Trp Ser Leu Gly Leu Ser Ile Leu Glu Met Ala Leu Gly
 545 550 555 560
 Arg Tyr Pro Tyr Pro Pro Glu Thr Tyr Asp Asn Ile Phe Ser Gln Leu
 565 570 575
 Ser Ala Ile Val Asp Gly Pro Pro Pro Arg Leu Pro Ser Asp Lys Phe
 580 585 590

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S r Ser Asp Ala Gln Asp Phe Val Ser Leu Cys Leu Gln Lys Ile Pro
 595 600 605
 Glu Arg Arg Pro Thr Tyr Ala Ala Leu Thr Glu His Pro Trp Leu Val
 610 615 620
 Lys Tyr Arg Asn Gln Asp Val His Met Ser Glu Tyr Ile Thr Glu Arg
 625 630 635 640
 Leu Glu Arg Arg Asn Lys Ile Leu Arg Glu Arg Gly Glu Asn Gly Leu
 645 650 655
 Ser Lys Asn Val Pro Ala Leu His Met Gly Gly Leu
 660 665

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TTYTAYGGNG CNTTYTTYAT HGA

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(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATBCTYTCNG GNGCCATKTA

20

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ASTYRYSASA SASASYS

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CLAIMS

What is claimed is:

1. A substantially pure human mitogen-activated protein kinase kinase (MKK) polypeptide having serine,
5 threonine, and tyrosine kinase activity, and phosphorylating human mitogen-activated protein (MAP) kinase p38.
2. A polypeptide of claim 1 comprising the amino acid sequence of SEQ ID NO:2.
- 10 3. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 2.
4. An isolated and purified polynucleotide sequence of claim 3 consisting of the sequence of SEQ ID NO:1 or degenerate variants thereof, or a polynucleotide
15 sequence fully complementary to the sequence of SEQ ID NO:1 or degenerate variants thereof.
5. An isolated and purified polynucleotide sequence of claim 3 consisting of a polynucleotide sequence that hybridizes under stringent hybridization
20 conditions to the sequence of SEQ ID NO:1.
6. A polypeptide of claim 1 comprising the amino acid sequence of SEQ ID NO:4.
7. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 6.
- 25 8. An isolated and purified polynucleotide sequence of claim 3 consisting of the sequence of SEQ ID NO:3 or degenerate variants thereof, or a polynucleotide

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sequence fully complementary to the sequence of SEQ ID NO:3 or degenerate variants thereof.

9. An isolated and purified polynucleotide sequence of claim 7 consisting of a polynucleotide
5 sequence that hybridizes under stringent hybridization conditions to the sequence of SEQ ID NO:3.

10. A polypeptide of claim 1, further characterized in that said polypeptide phosphorylates human mitogen-activated protein (MAP) kinase JNK.

10 11. A polypeptide of claim 10 comprising the amino acid sequence of SEQ ID NO:6.

12. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 11.

13. An isolated and purified polynucleotide
15 sequence of claim 12 consisting of the sequence of SEQ ID NO:5 or degenerate variants thereof, or a polynucleotide sequence fully complementary to the sequence of SEQ ID NO:5 or degenerate variants thereof.

14. An isolated and purified polynucleotide
20 sequence of claim 12 consisting of a polynucleotide sequence that hybridizes under stringent hybridization conditions to the sequence of SEQ ID NO:5.

15. A polypeptide of claim 10 comprising an amino acid sequence of SEQ ID NO:8.

25 16. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 15.

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17. An isolated and purified polynucleotide sequence of claim 16 consisting of the sequence of SEQ ID NO:7 or degenerate variants thereof, or a polynucleotide sequence fully complementary to the sequence of SEQ ID NO:7 or degenerate variants thereof.

18. An isolated and purified polynucleotide sequence of claim 16 consisting of a polynucleotide sequence that hybridizes under stringent hybridization conditions to the sequence of SEQ ID NO:7.

19. A polypeptide of claim 10 comprising the amino acid sequence of SEQ ID NO:10.

20. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 19.

21. An isolated and purified polynucleotide sequence of claim 20 consisting of the sequence of SEQ ID NO:9 or degenerate variants thereof, or a polynucleotide sequence fully complementary to the sequence of SEQ ID NO:9 or degenerate variants thereof.

22. A recombinant expression vector comprising a polynucleotide sequence of any one of claims 3, 7, 12, 16, or 20.

23. A recombinant host cell comprising a polynucleotide sequence of any one of claims 3, 7, 12, 16, or 20.

24. A purified antibody which binds specifically to a polypeptide of any one of claims 1, 2, 6, 10, 11, 15, or 19.

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25. A method of measuring the activity of a mitogen-activated protein kinase kinase (MKK) in a biological test sample, said method comprising:

- a) incubating said test sample with an MKK
5 substrate for the MKK polypeptide of claim 1 and labeled phosphate under conditions sufficient to allow phosphorylation of said substrate, and
- b) determining the rate of incorporation of
labeled phosphate into said substrate, wherein said rate
10 of incorporation is a measure of MKK activity.

26. A method of claim 25, wherein said MKK substrate is selected from the group consisting of p38 and JNK MAP kinases, activating transcription factor-2 (ATF2), ATFa, cAMP response element binding protein (CRE-
15 BP), and c-Jun.

27. A method of claim 25, wherein said biological test sample is fluid, cells, or tissue obtained from a mammal.

28. A method for measuring the synthesis of MKK
20 in a biological test sample, comprising the steps of:

- a) fractionating proteins present in said sample by gel electrophoresis;
- b) transferring the proteins onto a membrane; and
- c) probing the proteins with a labeled antibody
25 specific to a MKK polypeptide of claim 1, wherein the level of MKK synthesis is determined by the amount of bound labeled antibody.

29. A method for measuring the level of expression of MKK in a test sample, comprising the steps
30 of:

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- a) isolating polyadenylated RNA from the test sample;
- b) incubating polyadenylated RNA with a polynucleotide probe specific for a MKK polypeptide of claim 1;
- c) determining the amount of said probe hybridized said polyadenylated RNA, wherein the level of expression of MKK is directly related to the amount of MKK probe hybridized to said RNA.

30. A method for identifying a reagent which modulates MKK synthesis, said method comprising:
- a) using the method of claim 28;
 - b) comparing the effect of said reagent on MKK synthesis relative to a control, wherein a reagent able to modulate MKK synthesis is identified.

31. A method of claim 30 wherein said MKK substrate is one or more of p38, JNK, ATF2, ATFa, CRE-BPa, and c-Jun.

32. A method of claim 30 wherein said modulation is inhibition of MKK synthesis.

33. A substantially pure human mitogen-activated protein kinase kinase (MKK) polypeptide of any one of claims 1, 2, 6, 10, 11, 15, or 19 for use in treating an MKK-mediated disorder.

34. The polypeptide of claim 33, wherein said MKK-mediated disorder is selected from the group consisting of ischemic heart disease, kidney failure, oxidative liver damage, respiratory distress syndrome, heat and radiation burns, septic shock, rheumatoid

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arthritis, autoimmune disorders, and inflammatory diseases.

35. The use of a polypeptide of claim 33 for the manufacture of a medicament for the treatment of an MKK-5 mediated disorder.

36. A kit useful for the detection of MKK, said kit comprising a buffer and a reagent which binds to a MKK polypeptide of any one of claims 1, 2, 6, 10, 11, 15, or 19, wherein a sample to be tested is mixed with said 10 buffer and said reagent, and wherein said reagent is labeled.

37. A kit of claim 36, wherein said reagent is an antibody that specifically binds MKK.

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101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

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FIG. 1

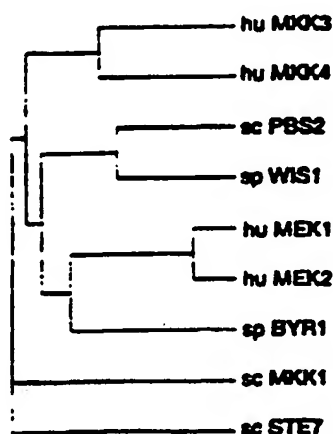


FIG. 2

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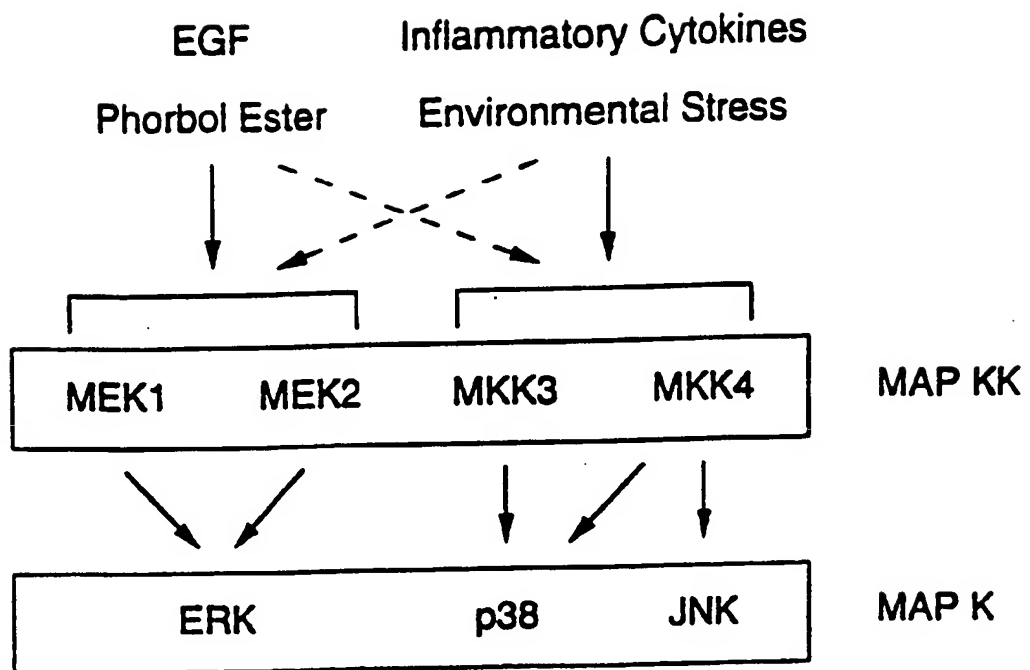


FIG. 3

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FIG. 4

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      5   10   15   20   25   30   35   40   45   50   55   60
      *   *   *   *   *   *   *   *   *   *   *   *
TGGCTGGCAA TGGCCTTGCT GACCTCGAGC CGGGCCACG TGGGGACCTT TGGAGCACAG
ACCGACCGTT ACCGGAACGA CTGGAGCTCG GCCCGGGTGC ACCCCTGGAA ACCTCGTGTC

      65   70   75   80   85   90   95  100  105  110  115  120
      *   *   *   *   *   *   *   *   *   *   *   *
CCTACGATCC TGGTGCAAGG CCGGTGGATG CAGAGGCCAG TCCATATACC ACCCAGGCCT
GGATGCTAGG ACCACGTTCC GGCCACCTAC GTCTCCGGTC AGGTATATGG TGGGTCCGGA

      125  130  135  140  145  150  155  160  165  170  175  180
      *   *   *   *   *   *   *   *   *   *   *   *
GCGAGGAGCG TGGTCCCCAC CCATCCAGCC CATATGTGCA AGTGGCCTTG ACAGAGAGGC
CGCTCCTCGC ACCAGGGGTG GGTAGGTCGG GTATACACGT TCACGGGAAC TGCTCTCCG

      185  190  195  200  205  210  215  220  225  230  235  240
      *   *   *   *   *   *   *   *   *   *   *   *
TGGTCATATC CATGGTGACC ATTTATGGGC CACAACAGGT CCCCATCTGC GCAGTGAACC
ACCAATATAG GTACCACTGG TAAATACCCG GTGTGTCCA GGGGTAGACG CGTCACTTGG

      245  250  255  260  265  270  275  280  285  290  295  300
      *   *   *   *   *   *   *   *   *   *   *   *
CTGTGCTGAG CACCTTGACG ACGTGATCTT GCTTCGTCTT GCAGCACTGT GCGGGGCAGG
GACACGACTC GTGGAACGTC TGCACTAGAA CGAAGCAGGA CGTCGTGACA CGCCCCGTCC

      305  310  315  320  325  330  335  340  345  350  355
      *   *   *   *   *   *   *   *   *   *   *
AAAATCCAAG AGGAAGAAGG ATCTACGGAT ATCTTGC ATG TCC AAG CCA CCC GCA
TTTTAGGTTC TCCTTCTTCC TAGATGCCTA TAGGACG TAC AGG TTC GGT GGG CGT
                                         Met Ser Lys Pro Pro Ala>

      360  365  370  375  380  385  390  395  400
      *   *   *   *   *   *   *   *   *
CCC AAC CCC ACA CCC CCC CGG AAC CTG GAC TCC CGG ACC TTC ATC ACC
GGG TTG GGG TGT GGG GGG GCC TTG GAC CTG AGG GCC TGG AAG TAG TGG
Pro Asn Pro Thr Pro Pro Arg Asn Leu Asp Ser Arg Thr Phe Ile Thr>

      405  410  415  420  425  430  435  440  445  450
      *   *   *   *   *   *   *   *   *   *
ATT GGA GAC AGA AAC TTT GAG GTG GAG GCT GAT GAC TTG GTG ACC ATC
TAA CCT CTG TCT TTG AAA CTC CAC CTC CGA CTA CTG AAC CAC TGG TAG
Ile Gly Asp Arg Asn Phe Glu Val Glu Ala Asp Asp Leu Val Thr Ile>

      455  460  465  470  475  480  485  490  495
      *   *   *   *   *   *   *   *   *
TCA GAA CTG GGC CGT GGA GCC TAT GGG GTG GTA GAG AAG GTG CGG CAC
AGT CTT GAC CCG GCA CCT CGG ATA CCC CAC CAT CTC TTC CAC GCC GTG
Ser Glu Leu Gly Arg Gly Ala Tyr Gly Val Val Glu Lys Val Arg His>

      500  505  510  515  520  525  530  535  540  545
      *   *   *   *   *   *   *   *   *   *
GCC CAG AGC GGC ACC ATC ATG GCC GTG AAG CGG ATC CGG GCC ACC GTG
CGG GTC TCG CCG TGG TAG TAC CGG CAC TTC GCC TAG GCC CGG TGG CAC
Ala Gln Ser Gly Thr Ile Met Ala Val Lys Arg Ile Arg Ala Thr Val>

      550  555  560  565  570  575  580  585  590  595
      *   *   *   *   *   *   *   *   *   *
AAC TCA CAG GAG CAG AAG CGG CTG CTC ATG GAC CTG GAC ATC AAC ATG
TTG AGT GTC CTC GTC TTC GCC GAC GAG TAC CTG GAC CTG TAG TTG TAC
Asn Ser Gln Glu Gln Lys Arg Leu Leu Met Asp Leu Asp Il Asn Met>

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FIG. 4 - CONT'D

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      600      605      610      615      620      625      630      635      640
      *      *      *      *      *      *      *      *      *
CGC ACG GTC GAC TGT TTC TAC ACT GTC ACC TTC TAC GGG GCA CTA TTC
GCG TGC CAG CTG ACA AAG ATG TGA CAG TGG AAG ATG CCC CGT GAT AAG
Arg Thr Val Asp Cys Phe Tyr Thr Val Thr Phe Tyr Gly Ala Leu Phe>

645      650      655      660      665      670      675      680      685      690
      *      *      *      *      *      *      *      *      *
AGA GAG GGA GAC GTG TGG ATC TGC ATG GAG CTC ATG GAC ACA TCC TTG
TCT CTC CCT CTG CAC ACC TAG ACG TAC CTC GAG TAC CTG TGT AGG AAC
Arg Glu Gly Asp Val Trp Ile Cys Met Glu Leu Met Asp Thr Ser Leu>

      695      700      705      710      715      720      725      730      735
      *      *      *      *      *      *      *      *      *
GAC AAG TTC TAC CGG AAG GTG CTG GAT AAA AAC ATG ACA ATT CCA GAG
CTG TTC AAG ATG GCC TTC CAC GAC CTA TTT TTG TAC TGT TAA GGT CTC
Asp Lys Phe Tyr Arg Lys Val Leu Asp Lys Asn Met Thr Ile Pro Glu>

740      745      750      755      760      765      770      775      780      785
      *      *      *      *      *      *      *      *      *
GAC ATC CTT GGG GAG ATT GCT GTG TCT ATC GTG CGG GCC CTG GAG CAT
CTG TAG GAA CCC CTC TAA CGA CAC AGA TAG CAC GCC CGG GAC CTC GTA
Asp Ile Leu Gly Glu Ile Ala Val Ser Ile Val Arg Ala Leu Glu His>

790      795      800      805      810      815      820      825      830      835
      *      *      *      *      *      *      *      *      *
CTG CAC AGC AAG CTG TCG GTG ATC CAC AGA GAT GTG AAG CCC TCC AAT
GAC GTG TCG TTC GAC AGC CAC TAG GTG TCT CTA CAC TTC GGG AGG TTA
Leu His Ser Lys Leu Ser Val Ile His Arg Asp Val Lys Pro Ser Asn>

      840      845      850      855      860      865      870      875      880
      *      *      *      *      *      *      *      *      *
GTC CTT ATC AAC AAG GAG GGC CAT GTG AAG ATG TGT GAC TTT GGC ATC
CAG GAA TAG TTG TTC CTC CCG GTA CAC TTC TAC ACA CTG AAA CCG TAG
Val Leu Ile Asn Lys Glu Gly His Val Lys Met Cys Asp Phe Gly Ile>

885      890      895      900      905      910      915      920      925      930
      *      *      *      *      *      *      *      *      *
AGT GGC TAC TTG GTG GAC TCT GTG GCC AAG ACG ATG GAT GCC GGC TGC
TCA CCG ATG AAC CAC CTG AGA CAC CCG TTC TGC TAC CTA CCG CCG ACG
Ser Gly Tyr Leu Val Asp Ser Val Ala Lys Thr Met Asp Ala Gly Cys>

      935      940      945      950      955      960      965      970      975
      *      *      *      *      *      *      *      *      *
AAG CCC TAC ATG GCC CCT GAG AGG ATC AAC CCA GAG CTG AAC CAG AAG
TTC GGG ATG TAC CGG GGA CTC TCC TAG TTG GGT CTC GAC TTG GTC TTC
Lys Pro Tyr Met Ala Pro Glu Arg Ile Asn Pro Glu Leu Asn Gln Lys>

980      985      990      995      1000      1005      1010      1015      1020      1025
      *      *      *      *      *      *      *      *      *
GCC TAC AAT GTC AAG TCC GAC GTC TGG AGC CTG GGC ATC ACC ATG ATT
CCG ATG TTA CAG TTC AGG CTG CAG ACC TCG GAC CCG TAG TGG TAC TAA
Gly Tyr Asn Val Lys Ser Asp Val Trp Ser Leu Gly Ile Thr Met Ile>

1030      1035      1040      1045      1050      1055      1060      1065      1070      1075
      *      *      *      *      *      *      *      *      *
GAG ATG GCC ATC CTG CCG TTC CCT TAC GAG TCC TGG GGG ACC CCG TTC
CTC TAC CCG TAG GAC GAC AAG GGA ATG CTC AGG ACC CCC TGG GGC AAG
Glu Met Ala Ile Leu Arg Phe Pro Tyr Glu Ser Trp Gly Thr Pr Phe>

1080      1085      1090      1095      1100      1105      1110      1115      1120

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FIG. 4 - CONT'D

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      *           *           *           *           *
CAG CAG CTG AAG CAG GTG GTG GAG GAG CCG TCC CCC CAG CTC CCA GCC
GTC GTC GAC TTC GTC CAC CAC CTC CTC GGC AGG GGG GTC GAG GGT CCG
Gln Gln Leu Lys Gln Val Val Glu Glu Pro Ser Pro Gln Leu Pro Ala>

1125  1130  1135  1140  1145  1150  1155  1160  1165  1170
      *           *           *           *           *
GAC CGT TTC TCC CCC GAG TTT GTG GAC TTC ACT GCT CAG TGC CTG AGG
CTG GCA AAG AGG GGG CTC AAA CAC CTG AAG TGA CGA GTC ACG GAC TCC
Asp Arg Phe Ser Pro Glu Phe Val Asp Phe Thr Ala Gln Cys Leu Arg>

1175  1180  1185  1190  1195  1200  1205  1210  1215
      *           *           *           *           *
AAG AAC CCC GCA GAG CGT ATG AGC TAC CTG GAG CTG ATG GAG CAC CCC
TTC TTG GGG CGT CTC GCA TAC TCG ATG GAC CTC GAC TAC CTC GTG GGG
Lys Asn Pro Ala Glu Arg Met Ser Tyr Leu Glu Leu Met Glu His Pro>

1220  1225  1230  1235  1240  1245  1250  1255  1260  1265
      *           *           *           *           *
TTC TTC ACC TTG CAC AAA ACC AAG AAG ACG GAC ATT GCT GCC TTC GTG
AAG AAG TGG AAC GTG TTT TGG TTC TTC TGC CTG TAA CGA CCG AAG CAC
Phe Phe Thr Leu His Lys Thr Lys Lys Thr Asp Ile Ala Ala Phe Val>

1270  1275  1280  1285  1290  1295  1300  1305  1310  1315  1320
      *           *           *           *           *
AAG AAG ATC CTG GGA GAA GAC TCA TAGGGGCTG GGCCTCGGAC CCCACTCCGG
TTC TTC TAG GAC CCT CTT CTG AGT ATCCCCGAC CCGGAGCCTG GGGTGAGGCC
Lys Lys Ile Leu Gly Glu Asp Ser> (SEQ ID NO:2)

1325  1330  1335  1340  1345  1350  1355  1360  1365  1370  1375  1380
      *           *           *           *           *
CCCTCCAGAG CCCACAGCC CCATCTGCGG GGGCAGTGCT CACCCACACC ATAAGCTACT
GGGAGGTCTC GGGGTGTCGG GGTAGACGCC CCCGTCACGA GTGGGTGTGG TATTCGATGA

1385  1390  1395  1400  1405  1410  1415  1420  1425  1430  1435  1440
      *           *           *           *           *
GCCATCCTGG CCCAGGGCAT CTGGGAGGAA CCGAGGGGGC TGCTCCACCC TGGCTCTGTG
CGGTAGGACC GGGTCCCGTA GACCCTCCTT GGCTCCCCCG ACGAGGGTGG ACCGAGACAC

1445  1450  1455  1460  1465  1470  1475  1480  1485  1490  1495  1500
      *           *           *           *           *
GCCAGCCATT TGTCCCAAGT GCCAAAGAAG CAGACCATTG GGGCTCCACG CCAGGCCCTT
CGCTCGGTAA ACAGGGTTCA CGGTTTCTTC GTCTGGTAAC CCCGAGGGTC GGTCCGGGAA

1505  1510  1515  1520  1525  1530  1535  1540  1545  1550  1555  1560
      *           *           *           *           *
GTCGGCCCCA CCAGTGCCTC TCCCTGCTGC TCCTAGGACC CGTCTCCAGC TGCTGAGATC
CAGCCGGGGT GGTACCGGAG AGGGACGACG AGGATCCTGG GCAGAGGTGC ACGACTCTAG

1565  1570  1575  1580  1585  1590  1595  1600  1605  1610  1615  1620
      *           *           *           *           *
CTGGACTGAG GGGCCTGGA TGCCCCCTGT GGATGCTGCT GCCCCTGCAC AGCAGGCTGC
GACCTGACTC CCCCAGACCT ACGGGGGACA CCTACGACGA CCGGGACGTG TCGTCCGACG

1625  1630  1635  1640  1645  1650  1655  1660  1665  1670  1675  1680
      *           *           *           *           *
CAGTGCTTGG GTGGATGGGC CACCGCCTTG CCCAGCCTGG ATGCCATCCA AGTTGTATAT
GTCACGGACC CACCTACCCG GTGGCGGAAC GGGTCGGACC TACGGTAGGT TCAACATATA

1685  1690  1695  1700  1705  1710  1715  1720  1725  1730  1735  1740
      *           *           *           *           *
TTTTTTAATC TCTCGACTGA ATGGACTTTG CACACTTTGG CCCAGGGTGG CCACACCTCT

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FIG. 4 - CONT'D

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AAAAAATTAG AGAGCTGACT TACCTGAAAC GTGTGAAACC GGGTCCCACC GGTGTGGAGA
1745 1750 1755 1760 1765 1770 1775 1780 1785 1790 1795 1800
ATCCCGGCTT TGGTGCGGGG TACACAAGAG GGGATGAGTT GTGTGAATAC CCCAAGACTC
TAGGGCCGAA ACCACGCCCC ATGTGTTCTC CCCTACTCAA CACACTTATG GGGTTCTGAG
1805 1810 1815 1820 1825 1830 1835 1840 1845 1850 1855 1860
CCATGAGGGA GATGCCATGA GCCGCCCAAG GCCTTCCCCT GGCCTGGCA AACAGGGCCT
GGTACTCCCT CTACGGTACT CGGCGGGTTC CGGAAGGGGA CCGTGACCGT TTGTCCCGGA
1865 1870 1875 1880 1885 1890 1895 1900 1905 1910 1915 1920
CTGCGGAGCA CACTGGCTCA CCCAGTCCTG CCCGCCACCG TTATCGGTGT CATTCACCTT
GACGCCCTCGT GTGACCGAGT GGGTCAGGAC GGGCGGTGGC AATAGCCACA GTAAGTGGA
1925 1930 1935 1940 1945 1950 1955 1960 1965 1970 1975 1980
TCGTGTTTTT TTTAATTTAT CCTCTGTTGA TTTTTCTTT TGCTTTATGG GTTTGGCTTG
AGCACAAAAA AAATTAAATA GGAGACAAC TAAAAAGAAA ACGAAATACC CAAACCGAAC
1985 1990 1995 2000 2005 2010 2015 2020 2025 2030
TTTTTCTTGC ATGGTTTGA GCTGATCGCT TCTCCCCCAC CCCCTAGGGG (SEQ ID NO: 1)
AAAAAGAACG TACCAAACCT CGACTAGCGA AGAGGGGGTG GGGGATCCCC

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FIG. 5

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      5   10   15   20   25   30   35   40   45   50   55   60
      *   *   *   *   *   *   *   *   *   *   *   *
TAGCTGCAGC ACAGCCTTCC CTAACGTTGC AACTGGGGGA AAAATCACTT TCCAGTCTGT
ATCGACGTCG TGTGGAAGG GATTGCAACG TTGACCCCT TTTTAGTGAA AGGTCAGACA

      65   70   75   80   85   90   95  100  105  110  115  120
      *   *   *   *   *   *   *   *   *   *   *   *
TTTGAAGGT GTGCATTTCC ATCTTGATTC CCTGAAAGTC CATCTGCTGC ATCGGTCAAG
AAACGTTCCA CACGTAAAGG TAGAACTAAG GGACTTTTCAG GTAGACGACG TAGCCAGTTC

      125  130  135  140  145  150  155  160  165  170  175  180
      *   *   *   *   *   *   *   *   *   *   *   *
AGAAACTCCA CTTGCATGAA GATTGCACGC CTGCAGCTTG CATCTTTGTT GCAAAACTAG
TCTTTGAGGT GAACGTACTT CTAACGTGCG GACGTCGAAC GTAGAAACAA CGTTTTGATC

      185  190  195  200  205  210  215  220  225  230  235  240
      *   *   *   *   *   *   *   *   *   *   *   *
CTACAGAAGA GAAGCAAGGC AAAGTCTTTT GTGCTCCCT CCCCCATCAA AGGAAAGGGG
GATGTCTTCT CTTGTTCCG TTTCAGAAAA CACGAGGGGA GGGGGTAGTT TCCTTTCCCC

      245  250  255  260  265  270  275  280  285
      *   *   *   *   *   *   *   *   *
AAA ATG TCT CAG TCG AAA GGC AAG AAG CGA AAC CCT GGC CTT AAA ATT
TTT TAC AGA GTC AGC TTT CCG TTC TTC GCT TTG GGA CCG GAA TTT TAA
Met Ser Gln Ser Lys Gly Lys Lys Arg Asn Pro Gly Leu Lys Ile>

      290  295  300  305  310  315  320  325  330  335
      *   *   *   *   *   *   *   *   *   *
CCA AAA GAA GCA TTT GAA CAA CCT CAG ACC AGT TCC ACA CCA CCT AGA
GGT TTT CTT CGT AAA CTT GTT GGA GTC TGG TCA AGG TGT GGT GGA TCT
Pro Lys Glu Ala Phe Glu Gln Pro Gln Thr Ser Ser Thr Pro Pro Arg>

      340  345  350  355  360  365  370  375  380
      *   *   *   *   *   *   *   *   *
GAT TTA GAC TCC AAG GCT TGC ATT TCT ATT GGA AAT CAG AAC TTT GAG
CTA AAT CTG AGG TTC CGA ACG TAA AGA TAA CCT TTA GTC TTG AAA CTC
Asp Leu Asp Ser Lys Ala Cys Ile Ser Ile Gly Asn Gln Asn Phe Glu>

      385  390  395  400  405  410  415  420  425  430
      *   *   *   *   *   *   *   *   *   *
GTG AAG GCA GAT GAC CTG GAG CCT ATA ATG GAA CTG GGA CGA GGT GCG
CAC TTC CGT CTA CTG GAC CTC GGA TAT TAC CTT GAC CCT GCT CCA CGC
Val Lys Ala Asp Asp Leu Glu Pro Ile Met Glu Leu Gly Arg Gly Ala>

      435  440  445  450  455  460  465  470  475  480
      *   *   *   *   *   *   *   *   *   *
TAC GGG GTG GTG GAG AAG ATG CGG CAC GTG CCC AGC GGG CAG ATC ATG
ATG CCC CAC CAC CTC TTC TAC GCC GTG CAC GGG TCG CCC GTC TAG TAC
Tyr Gly Val Val Glu Lys Met Arg His Val Pro Ser Gly Gln Ile Met>

      485  490  495  500  505  510  515  520  525
      *   *   *   *   *   *   *   *   *
GCA GTG AAG CGG ATC CGA GCC ACA GTA AAT AGC CAG GAA CAG AAA CGG
CGT CAC TTC GCC TAG GCT CGG TGT CAT TTA TCG GTC CTT GTC TTT GCC
Ala Val Lys Arg Ile Arg Ala Thr Val Asn Ser Gln Glu Gln Lys Arg>

      530  535  540  545  550  555  560  565  570  575
      *   *   *   *   *   *   *   *   *   *
CTA CTG ATG GAT TTG GAT ATT TCC ATG AGG ACG GTG GAC TGT CCA TTC
GAT GAC TAC CTA AAC CTA TAA AGG TAC TCC TGC CAC CTG ACA GGT AAG

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FIG. 5 - CONT'D

Leu Leu Met Asp Leu Asp Ile Ser Met Arg Thr Val Asp Cys Pro Phe>
 580 585 590 595 600 605 610 615 620
 ACT GTC ACC TTT TAT GGC GCA CTG TTT CGG GAG GGT GAT GTG TGG ATC
 TGA CAG TGG AAA ATA CCG CGT GAC AAA GCC CTC CCA CTA CAC ACC TAG
 Thr Val Thr Phe Tyr Gly Ala Leu Phe Arg Glu Gly Asp Val Trp Ile>
 625 630 635 640 645 650 655 660 665 670
 TGC ATG GAG CTC ATG GAT ACA TCA CTA GAT AAA TTC TAC AAA CAA GTT
 ACG TAC CTC GAG TAC CTA TGT AGT GAT CTA TTT AAG ATG TTT GTT CAA
 Cys Met Glu Leu Met Asp Thr Ser Leu Asp Lys Phe Tyr Lys Gln Val>
 675 680 685 690 695 700 705 710 715 720
 ATT GAT AAA GGC CAG ACA ATT CCA GAG GAC ATC TTA GGG AAA ATA GCA
 TAA CTA TTT CCG GTC TGT TAA GGT CTC CTG TAG AAT CCC TTT TAT CGT
 Ile Asp Lys Gly Gln Thr Ile Pro Glu Asp Ile Leu Gly Lys Ile Ala>
 725 730 735 740 745 750 755 760 765
 GTT TCT ATT GTA AAA GCA TTA GAA CAT TTA CAT AGT AAG CTG TCT GTC
 CAA AGA TAA CAT TTT CGT AAT CTT GTA AAT GTA TCA TTC GAC AGA CAG
 Val Ser Ile Val Lys Ala Leu Glu His Leu His Ser Lys Leu Ser Val>
 770 775 780 785 790 795 800 805 810 815
 ATT CAC AGA GAC GTC AAG CCT TCT AAT GTA CTC ATC AAT GCT CTC GGT
 TAA GTG TCT CTG CAG TTC GGA AGA TTA CAT GAG TAG TTA CGA GAG CCA
 Ile His Arg Asp Val Lys Pro Ser Asn Val Leu Ile Asn Ala Leu Gly>
 820 825 830 835 840 845 850 855 860
 CAA GTG AAG ATG TGC GAT TTT GGA ATC AGT GGC TAC TTG GTG GAC TCT
 GTT CAC TTC TAC ACG CTA AAA CCT TAG TCA CCG ATG AAC CAC CTG AGA
 Gln Val Lys Met Cys Asp Phe Gly Ile Ser Gly Tyr Leu Val Asp Ser>
 865 870 875 880 885 890 895 900 905 910
 GTT GCT AAA ACA ATT GAT GCA GGT TGC AAA CCA TAC ATG GCC CCT GAA
 CAA CGA TTT TGT TAA CTA CGT CCA ACG TTT GGT ATG TAC CGG GGA CTT
 Val Ala Lys Thr Ile Asp Ala Gly Cys Lys Pro Tyr Met Ala Pro Glu>
 915 920 925 930 935 940 945 950 955 960
 AGA ATA AAC CCA GAG CTC AAC CAG AAG GGA TAC AGT GTG AAG TCT GAC
 TCT TAT TTG GGT CTC GAG TTG GTC TTC CCT ATG TCA CAC TTC AGA CTG
 Arg Ile Asn Pro Glu Leu Asn Gln Lys Gly Tyr Ser Val Lys Ser Asp>
 965 970 975 980 985 990 995 1000 1005
 ATT TGG AGT CTG GGC ATC ACG ATG ATT GAG TTG GCC ATC CTT CGA TTT
 TAA ACC TCA GAC CCG TAG TGC TAC TAA CTC AAC CGG TAG GAA GCT AAA
 Ile Trp Ser Leu Gly Ile Thr Met Ile Glu Leu Ala Ile Leu Arg Phe>
 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055
 CCC TAT GAT TCA TGG GGA ACT CCA TTT CAG CAG CTC AAA CAG GTG GTA
 GGG ATA CTA AGT ACC CCT TGA GGT AAA GTC GTC GAG TTT GTC CAC CAT
 Pro Tyr Asp Ser Trp Gly Thr Pr Phe Gln Gln Leu Lys Gln Val Val>

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FIG. 5 - CONT'D

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1060 1065 1070 1075 1080 1085 1090 1095 1100
*      *      *      *      *      *      *      *
GAG GAG CCA TCG CCA CAA CTC CCA GCA GAC AAG TTC TCT GCA GAG TTT
CTC CTC GGT AGC GGT GTT GAG GGT CGT CTG TTC AAG AGA CGT CTC AAA
Glu Glu Pro Ser Pro Gln Leu Pro Ala Asp Lys Phe Ser Ala Glu Phe>

1105 1110 1115 1120 1125 1130 1135 1140 1145 1150
*      *      *      *      *      *      *      *
GTT GAC TTT ACC TCA CAG TGC TTA AAG AAG AAT TCC AAA GAA CGG CCT
CAA CTG AAA TGG AGT GTC ACG AAT TTC TTC TTA AGG TTT CTT GCC GGA
Val Asp Phe Thr Ser Gln Cys Leu Lys Lys Asn Ser Lys Glu Arg Pro>

1155 1160 1165 1170 1175 1180 1185 1190 1195 1200
*      *      *      *      *      *      *      *
ACA TAC CCA GAG CTA ATG CAA CAT CCA TTT TTC ACC CTA CAT GAA TCC
TGT ATG GGT CTC GAT TAC GTT GTA GGT AAA AAG TGG GAT GTA CTT AGG
Thr Tyr Pro Glu Leu Met Gln His Pro Phe Phe Thr Leu His Glu Ser>

1205 1210 1215 1220 1225 1230 1235 1240 1245 1250
*      *      *      *      *      *      *      *
AAA GGA ACA GAT GTG GCA TCT TTT GTA AAA CTG ATT CTT GGA GAC TAAAA
TTT CCT TGT CTA CAC CGT AGA AAA CAT TTT GAC TAA GAA CCT CTG ATTTT
Lys Gly Thr Asp Val Ala Ser Phe Val Lys Leu Ile Leu Gly Asp> (SEQ ID NO:4)

1255 1260 1265 1270 1275 1280 1285 1290 1295 1300 1305 1310
*      *      *      *      *      *      *      *
AGCAGTGGAC TTAATCGGTT GACCCCTACTG TGGATTGGTG GGTTCGGGG TGAAGCAAGT
TCGTCACCTG AATTAGCCAA CTGGGATGAC ACCTAACCAC CCAAAGCCCC ACTTCGTTCA

1315 1320 1325 1330 1335 1340 1345 1350 1355 1360 1365 1370
*      *      *      *      *      *      *      *
TCACTACAGC ATCAATAGAA AGTCATCTTT GAGATAATTT AACCCCTGCCT CTCAGAGGGT
AGTGATGTCG TAGTTATCTT TCAGTAGAAA CTCTATTAAA TTGGGACGGA GAGTCTCCCA

1375 1380 1385 1390 1395 1400 1405 1410 1415 1420 1425 1430
*      *      *      *      *      *      *      *
TTTCTCTCCC AATTTTCTTT TTAATCCCC TCTTAAGGGG GCCTTGAAT CTATAGTATA
AAAGAGAGGG TTAAGAGAAA AATGAGGGGG AGAATTCCCC CGGAACCTTA GATATCATAT

1435 1440 1445 1450 1455 1460 1465 1470 1475 1480 1485 1490
*      *      *      *      *      *      *      *
GAATGAACTG TCTAGATGGA TGAATTATGA TAAAGGCTTA GGACTTCAAA AGGTGATTAA
CTTACTTGAC AGATCTACCT ACTTAATACT ATTTCCGAAT CCTGAAGTTT TCCACTAATT

1495 1500 1505 1510 1515 1520 1525 1530 1535 1540 1545 1550
*      *      *      *      *      *      *      *
ATATTTAATG ATGTGTCATA TGAGTCCTCA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
TATAAATTAC TACACAGTAT ACTCAGGAGT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT

1555 1560 1565 1570 1575 1580 1585 1590 1595 1600
*      *      *      *      *      *      *      *
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AA (SEQ ID NO:3)
TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TT

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FIG. 6

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      5    10    15    20    25    30    35    40    45    50    55
      *      *      *      *      *      *      *      *      *
CTAGGGTCCC CGGCGCCAGG CCACCCGGCC GTCAGCAGC ATG CAG GGT AAA CGC AAA
GATCCAGGG GCCGCGGTCC GGTGGGCCGG CAGTCGTCG TAC GTC CCA TTT GCG TTT
                               Met Gln Gly Lys Arg Lys>

      60    65    70    75    80    85    90    95    100   105
      *      *      *      *      *      *      *      *      *
GCA CTG AAG TTG AAT TTT GCA AAT CCA CCT TTC AAA TCT ACA GCA AGG
CGT GAC TTC AAC TTA AAA CGT TTA GGT GGA AAG TTT AGA TGT CGT TCC
Ala Leu Lys Leu Asn Phe Ala Asn Pro Pro Phe Lys Ser Thr Ala Arg>

      110   115   120   125   130   135   140   145   150
      *     *     *     *     *     *     *     *     *
TTT ACT CTG AAT CCC AAT CCT ACA GGA GTT CAA AAC CCA CAC ATA GAG
AAA TGA GAC TTA GGG TTA GGA TGT CCT CAA GTT TTG GGT TAT CTC
Phe Thr Leu Asn Pro Asn Pro Thr Gly Val Gln Asn Pro His Ile Glu>

      155   160   165   170   175   180   185   190   195   200
      *     *     *     *     *     *     *     *     *
AGA CTG AGA ACA CAC AGC ATT GAG TCA TCA GGA AAA CTG AAG ATC TCC
TCT GAC TCT TGT GTG TCG TAA CTC AGT AGT CCT TTT GAC TTC TAG AGG
Arg Leu Arg Thr His Ser Ile Glu Ser Ser Gly Lys Leu Lys Ile Ser>

      205   210   215   220   225   230   235   240   245
      *     *     *     *     *     *     *     *     *
CCT GAA CAA CAC TGG GAT TTC ACT GCA GAG GAC TTG AAA GAC CTT GGA
GGA CTT GTT GTG ACC CTA AAG TGA CGT CTC CTG AAC TTT CTG GAA CCT
Pro Glu Gln His Trp Asp Phe Thr Ala Glu Asp Leu Lys Asp Leu Gly>

      250   255   260   265   270   275   280   285   290   295
      *     *     *     *     *     *     *     *     *
GAA ATT GGA CGA GGA GCT TAT GGT TCT GTC AAC AAA ATG GTC CAC AAA
CTT TAA CCT GCT CCT CGA ATA CCA AGA CAG TTG TTT TAC CAG GTG TTT
Glu Ile Gly Arg Gly Ala Tyr Gly Ser Val Asn Lys Met Val His Lys>

      300   305   310   315   320   325   330   335   340   345
      *     *     *     *     *     *     *     *     *
CCA AGT GGG CAA ATA ATG GCA GTT AAA AGA ATT CGG TCA ACA GTG GAT
GGT TCA CCC GTT TAT TAC CGT CAA TTT TCT TAA GCC AGT TGT CAC CTA
Pro Ser Gly Gln Ile Met Ala Val Lys Arg Ile Arg Ser Thr Val Asp>

      350   355   360   365   370   375   380   385   390
      *     *     *     *     *     *     *     *     *
GAA AAA GAA CAA AAA CAA CTT CTT ATG GAT TTG GAT GTA GTA ATG CGG
CTT TTT CTT GTT TTT GTT GAA GAA TAC CTA AAC CTA CAT CAT TAC GCC
Glu Lys Glu Gln Lys Gln Leu Leu Met Asp Leu Asp Val Val Met Arg>

      395   400   405   410   415   420   425   430   435   440
      *     *     *     *     *     *     *     *     *
AGT AGT GAT TGC CCA TAC ATT GTT CAG TTT TAT GGT GCA CTC TTC AGA
TCA TCA CTA ACG GGT ATG TAA CAA GTC AAA ATA CCA CGT GAG AAG TCT
Ser Ser Asp Cys Pro Tyr Ile Val Gln Phe Tyr Gly Ala Leu Phe Arg>

      445   450   455   460   465   470   475   480   485
      *     *     *     *     *     *     *     *     *
GAG GGT GAC TGT TGG ATC TGT ATG GAA CTC ATG TCT ACC TCG TTT GAT
CTC CCA CTG ACA ACC TAG ACA TAC CTT GAG TAC AGA TGG AGC AAA CTA
Glu Gly Asp Cys Trp Ile Cys Met Glu Leu Met Ser Thr Ser Phe Asp>

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FIG. 6 - CONT'D

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490   495   500   505   510   515   520   525   530   535
*       *       *       *       *       *       *       *
AAG TTT TAC AAA TAT GTA TAT AGT GTA TTA GAT GAT GTT ATT CCA GAA
TTC AAA ATG TTT ATA CAT ATA TCA CAT AAT CTA CTA CAA TAA GGT CTT
Lys Phe Tyr Lys Tyr Val Tyr Ser Val Leu Asp Asp Val Ile Pro Glu>

540   545   550   555   560   565   570   575   580   585
*       *       *       *       *       *       *       *
GAA ATT TTA GGC AAA ATC ACT TTA GCA ACT GTG AAA GCA CTA AAC CAC
CTT TAA AAT CCG TTT TAG TGA AAT CGT TGA CAC TTT CGT GAT TTG GTG
Glu Ile Leu Gly Lys Ile Thr Leu Ala Thr Val Lys Ala Leu Asn His>

590   595   600   605   610   615   620   625   630
*       *       *       *       *       *       *
TTA AAA GAA AAC TTG AAA ATT ATT CAC AGA GAT ATC AAA CCT TCC AAT
AAT TTT CTT TTG AAC TTT TAA TAA GTG TCT CTA TAG TTT GGA AGG TTA
Leu Lys Glu Asn Leu Lys Ile Ile His Arg Asp Ile Lys Pro Ser Asn>

635   640   645   650   655   660   665   670   675   680
*       *       *       *       *       *       *
ATT CTT CTG GAC AGA AGT GGA AAT ATT AAG CTC TGT GAC TTC GGC ATC
TAA GAA GAC CTG TCT TCA CCT TTA TAA TTC GAG ACA CTG AAG CCG TAG
Ile Leu Leu Asp Arg Ser Gly Asn Ile Lys Leu Cys Asp Phe Gly Ile>

685   690   695   700   705   710   715   720   725
*       *       *       *       *       *
AGT GGA CAG CTT GTG GAC TCT ATT GCC AAG ACA AGA GAT GCT GGC TGT
TCA CCT GTC GAA CAC CTG AGA TAA CGG TTC TGT TCT CTA CGA CCG ACA
Ser Gly Gln Leu Val Asp Ser Ile Ala Lys Thr Arg Asp Ala Gly Cys>

730   735   740   745   750   755   760   765   770   775
*       *       *       *       *       *
AGG CCA TAC ATG GCA CCT GAA AGA ATA GAC CCA AGC GCA TCA CGA CAA
TCC GGT ATG TAC CGT GGA CTT TCT TAT CTG GGT TCG CGT AGT GCT GTT
Arg Pro Tyr Met Ala Pro Glu Arg Ile Asp Pro Ser Ala Ser Arg Gln>

780   785   790   795   800   805   810   815   820   825
*       *       *       *       *       *
GGA TAT GAT GTC CGC TCT GAT GTC TGG AGT TTG GGG ATC ACA TTG TAT
CCT ATA CTA CAG GCG AGA CTA CAG ACC TCA AAC CCC TAG TGT AAC ATA
Gly Tyr Asp Val Arg Ser Asp Val Trp Ser Leu Gly Ile Thr Leu Tyr>

830   835   840   845   850   855   860   865   870
*       *       *       *       *       *
GAG TTG GCC ACA GGC CGA TTT CCT TAT CCA AAG TGG AAT AGT GTA TTT
CTC AAC CGG TGT CCG GCT AAA GGA ATA GGT TTC ACC TTA TCA CAT AAA
Glu Leu Ala Thr Gly Arg Phe Pro Tyr Pro Lys Trp Asn Ser Val Phe>

875   880   885   890   895   900   905   910   915   920
*       *       *       *       *       *
GAT CAA CTA ACA CAA GTC GTG AAA GGA GAT CCT CCG CAG CTG AGT AAT
CTA GTT GAT TGT GTT CAG CAC TTT CCT CTA GGA GGC GTC GAC TCA TTA
Asp Gln Leu Thr Gln Val Val Lys Gly Asp Pro Pro Gln Leu Ser Asn>

925   930   935   940   945   950   955   960   965
*       *       *       *       *       *
TCT GAG GAA AGG GAA TTC TCC CCG AGT TTC ATC AAC TTT GTC AAC TTG
AGA CTC CTT TCC CTT AAG AGG GGC TCA AAG TAG TTG AAA CAG TTG AAC
Ser Glu Glu Arg Glu Phe Ser Pro Ser Phe Ile Asn Phe Val Asn Leu>

970   975   980   985   990   995   1000  1005  1010  1015
*       *       *       *       *       *

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FIG. 6 - CONT'D

TGC CTT ACG AAG GAT GAA TCC AAA AGG CCA AAG TAT AAA GAG CTT CTG
 ACG GAA TGC TTC CTA CTT AGG TTT TCC GGT TTC ATA TTT CTC GAA GAC
 Cys Leu Thr Lys Asp Glu Ser Lys Arg Pro Lys Tyr Lys Glu Leu Leu>

1020 1025 1030 1035 1040 1045 1050 1055 1060 1065
 * * * * *
 AAA CAT CCC TTT ATT TTG ATG TAT GAA GAA CGT GCC GTT GAG GTC GCA
 TTT GTA GGG AAA TAA AAC TAC ATA CTT CTT GCA CGG CAA CTC CAG CGT
 Lys His Pro Phe Ile Leu Met Tyr Glu Glu Arg Ala Val Glu Val Ala>

1070 1075 1080 1085 1090 1095 1100 1105 1110
 * * * * *
 TGC TAT GTT TGT AAA ATC CTG GAT CAA ATG CCA GCT ACT CCC AGC TCT
 ACG ATA CAA ACA TTT TAG GAC CTA GTT TAC GGT CGA TGA GGG TCG AGA
 Cys Tyr Val Cys Lys Ile Leu Asp Gln Met Pro Ala Thr Pro Ser Ser>

1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 1170
 * * * * *
 CCC ATG TAT GTC GAT TG ATATCGYTGC TACATCAGAC TCTAGAAAAA AGGGCTGAGA
 GGG TAC ATA CAG CTA AC TATAGCRACG ATGTAGTCTG AGATCTTTTT TCCCGACTCT
 Pro Met Tyr Val Asp> (SEQ ID NO:6)

1175 1180 1185 1190 1195 1200 1205 1210 1215 1220 1225 1230
 * * * * *
 GGAAGCAAGA CGTAAAGAAT TTTCATCCCG TATCACAGTG TTTTATTGTC TCGCCAGAC
 CCTTCGTCTC GCATTTCTTA AAAGTAGGGC ATAGTGTACAC AAAAATAACG AGCGGGTCTG

1235 1240 1245 1250 1255 1260 1265 1270 1275 1280 1285 1290
 * * * * *
 ACCATGTGCA ATAAGATTGG TGTTCGTTTC CATCATGTCT GTATACTCCT GTCACCTAGA
 TGGTACACGT TATTCTAACC ACAAGCAAAG GTAGTACAGA CATATGAGGA CAGTGGATCT

1295 1300 1305 1310 1315 1320 1325 1330 1335 1340 1345 1350
 * * * * *
 ACGTGCATCC TTGTAATACC TGATTGATCA CACAGTGTTA GTGCTGGTCA GAGAGACCTC
 TGCACGTAGG AACATTATGG ACTAAGTAGT GTGTCACAAT CACGACCAGT CTCTCTGGAG

1355 1360 1365 1370 1375 1380 1385 1390 1395 1400 1405 1410
 * * * * *
 ATCCTGCTCT TTTGTGATGA ACATATTTCAT GAAATGTGGA AGTCAGTACG ATCAAGTTGT
 TAGGACGAGA AAACACTACT TGTATAAGTA CTTTACACCT TCAGTCATGC TAGTTCAACA

1415 1420 1425 1430 1435 1440 1445 1450 1455 1460 1465 1470
 * * * * *
 TGA CTGTGAT TAGATCACAT CTTAAATTCG TTTCTAGACT CAAAACCTGG AGATGCAGCT
 ACTGACACTA ATCTAGTGTA GAATTTAAGT AAAGATCTGA GTTTTGGACC TCTACGTCGA

1475 1480 1485 1490 1495 1500 1505 1510 1515 1520 1525 1530
 * * * * *
 ACTGGAATGG TGT TTTGTCA GACTTCACAA TCCTGGAAGG ACACAGTGAT GAATGTACTA
 TGACCTTACC ACAAACAGT CTGAAGGTTT AGGACCTTCC TGTGTCACTA CTTACATGAT

1535 1540 1545 1550 1555 1560 1565 1570 1575 1580 1585 1590
 * * * * *
 TATCTGAACA TAGAAACTCG GGCTTGAGTG AGAAGAGCTT GCACAGCCAA CGAGACACAT
 ATAGACTTGT ATCTTTGAGC CCGAACTCAC TCTTCTCGAA CGTGTGGTT GCTCTGTGTA

1595 1600 1605 1610 1615 1620 1625 1630 1635 1640 1645 1650
 * * * * *
 TGCCCTTCTGG AGCTGGGAGA CAAAGGAGGA ATTTACTTTT TCACCAAGT GCAATAGATT
 ACGGAAGACC TCGACCCTCT GTTCTCTCT TAAATGAAAG AAGTGGTTCA CGTTATCTAA

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FIG. 6 - CONT'D

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1655 1660 1665 1670 1675 1680 1685 1690 1695 1700 1705 1710
ACTGATGTGA TATTCTGTTG CTTTACAGTT ACAGTTGATG TTTGGGGATC GATGTGCTCA
TGACTACACT ATAAGACAAC GAAATGTCAA TGTCAACTAC AAACCCCTAG CTACACGAGT

1715 1720 1725 1730 1735 1740 1745 1750 1755 1760 1765 1770
GCCAAATTTT CTGTTTGAAA TATCATGTTA AATTAGAATG AATTTATCTT TACCAAAAAC
CGGTTTAAAG GACAACTTTT ATAGTACAAT TTAATCTTAC TTAAATAGAA ATGGTTTTGT

1775 1780 1785 1790 1795 1800 1805 1810 1815 1820 1825 1830
CATGTTGCGT TCAAAGAGGT GAACATTAAA ATATAGAGAC AGGACAGAAT GTGTTCTTTT
GTACAACGCA AGTTTCTCCA CTGTGAATTT TATATCTCTG TCCTGTCCTA CACAAGAAAA

1835 1840 1845 1850 1855 1860 1865 1870 1875 1880 1885 1890
CTCCTCTACC AGTCCTATTT TTCAATGGGA AGACTCAGGA GTCTGCCACT TGTCAAAGAA
GAGGAGATGG TCAGGATAAA AAGTTACCCCT TCTGAGTCCT CAGACGGTGA ACAGTTTCTT

1895 1900 1905 1910 1915 1920 1925 1930 1935 1940 1945 1950
GGTGCTGATC CTAAGAATTT TTCATTCTCA GAATTCGGTG TGCTGCCAAC TTGATGTTCC
CCACGACTAG GATTCTTAAA AAGTAAGAGT CTTAAGCCAC ACGACGGTTG AACTACAAGG

1955 1960 1965 1970 1975 1980 1985 1990 1995 2000 2005 2010
ACCTGCCACA AACCACCAGG ACTGAAAGAA GAAAACAGTA CAGAAGGCAA AGTTTACAGA
TGGACGGTGT TTGGTGGTCC TGACTTTCTT CTTTTGTCAT GTCTTCCGTT TCAAATGTCT

2015 2020 2025 2030 2035 2040 2045 2050 2055 2060 2065 2070
TGTTTTTAAT TCTAGTATTT TATCTGGAAC AACTTGTAGC AGCTATATAT TTCCCCTTGG
ACAAAAATTA AGATCATAAA ATAGACCTTG TTGAACATCG TCGATATATA AAGGGGAACC

2075 2080 2085 2090 2095 2100 2105 2110 2115 2120 2125 2130
TCCCAAGCCT GATACTTTAG CCATCATAAC TCACTAACAG GGAGAAGTAG CTAGTAGCAA
AGGGTTCGGA CTATGAAATC GGTAGTATTG AGTGATTGTC CCTCTTCATC GATCATCGTT

2135 2140 2145 2150 2155 2160 2165 2170 2175 2180 2185 2190
TGTGCCTTGA TTGATTAGAT AAAGATTCTT AGTAGGCAGC AAAAGACCAA ATCTCAGTTG
ACACGGAACT AACTAATCTA TTTCTAAAGA TCATCCGTCG TTTTCTGGTT TAGAGTCAAC

2195 2200 2205 2210 2215 2220 2225 2230 2235 2240 2245 2250
TTTGCTTCTT GCCATCACTG GTCCAGGTCT TCAGTTTCCG AATCTCTTTC CCTTCCCCTG
AAACGAAGAA CGGTAGTGAC CAGGTCCAGA AGTCAAAGGC TTAGAGAAAG GGAAGGGGAC

2255 2260 2265 2270 2275 2280 2285 2290 2295 2300 2305 2310
TGGTCTATTG TCGCTATGTG ACTTGCGCTT AATCCAATAT TTTGCCTTTT TTCTATATCA
ACCAGATAAC AGCGATACAC TGAACGCGAA TTAGGTTATA AAACGGAAAA AAGATAAGT

2315 2320 2325 2330 2335 2340 2345 2350 2355 2360 2365 2370
AAAAACCTTT ACAGTTAGCA GGGATGTTCC TTACCGAGGA TTTTTAACCC CCAATCTCTC
TTTTTGGAAA TGTCATCGT CCCTACAAGG AATGGCTCCT AAAAATTGGG GGTAGAGAG

2375 2380 2385 2390 2395 2400 2405 2410 2415 2420 2425 2430

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FIG. 6 - CONT'D

ATAATCGCTA	GTGTTTAAAA	GGCTAAGAAT	AGTGGGGCCC	AACCGATGTG	GTAGGTGATA
TATTAGCGAT	CACAAATTTT	CCGATTCTTA	TCACCCCGGG	TTGGCTACAC	CATCCACTAT
2435 2440	2445 2450	2455 2460	2465 2470	2475 2480	2485 2490
AAGAGGCATC	TTTTCTAGAG	ACACATITGA	CCAGATGAGG	ATCCGAAACG	GCAGCCTTTA
TTCTCCGTAG	AAAAGATCTC	TGTGTAACTT	GGTCTACTCC	TAGGCTTTGC	CGTCCGAAAT
2495 2500	2505 2510	2515 2520	2525 2530	2535 2540	2545 2550
CGTTCATCAC	CTGCTAGAAC	CTCTCGTAGT	CCATCACCAT	TTCTTGCCAT	TGGAATTCTA
GCAAGTAGTG	GACGATCTTG	GAGAGCATCA	GGTAGTGGTA	AAGAACCGTA	ACCTTAAGAT
2555 2560	2565 2570	2575 2580	2585 2590	2595 2600	2605 2610
CTGGAAAAAA	ATACAAAAAG	CAAAACAAAA	CCCTCAGCAC	TGTTACAAGA	GGCCATTTAA
GACCTTTTTT	TATGTTTTTC	GTTTGTGTTT	GGGAGTCGTG	ACAATGTTCT	CCGGTAAATT
2615 2620	2625 2630	2635 2640	2645 2650	2655 2660	2665 2670
GTATCTTGIG	CTTCTTCACT	TACCCATTAG	CCAGGTTCCT	ATTAGGTTTT	GCTTGGGCCT
CATAGAACAC	GAAGAAAGTA	ATGGGTAATC	GGTCCAAGAG	TAATCCAAAA	CGAACCCGGA
2675 2680	2685 2690	2695 2700	2705 2710	2715 2720	2725 2730
CCCTGGCACT	GAACCTTAGG	CTTGTATGA	CAGTGAAGCA	GCACTGTGAG	TGTTTCAAGC
GGGACCGTGA	CTTGGAAATC	GAAACATACT	GTCACTTCGT	CGTGACACTC	ACCAAGTTCC
2735 2740	2745 2750	2755 2760	2765 2770	2775 2780	2785 2790
ACACTGGAAT	ATAAAACAGT	CATGGCCTGA	GATGCAGGTG	ATGCCATTAC	AGAACCAAAT
TGTGACCTTA	TATTTTGTCA	GTACCGGACT	CTACGTCCAC	TACGGTAATG	TCTTGGTTTA
2795 2800	2805 2810	2815 2820	2825 2830	2835 2840	2845 2850
CGTGGCACGT	ATTGCTGTGT	CTCCTCTCAG	AGTGACAGTC	ATAAATACTG	TCAAACAATA
GCACCGTGCA	TAACGACACA	GAGGAGAGTC	TCACTGTGAG	TATTTATGAC	AGTTTGTAT
2855 2860	2865 2870	2875 2880	2885 2890	2895 2900	2905 2910
AAGGGAGAAT	GGTGTGTTTT	AAAGTCACAT	CCCTGTAAAT	TGCAGAATTC	AAAAGTGATT
TTCCCTCTTA	CCACGACAAA	TTTCAGTGTA	GGGACATTTA	ACGTCTTAAG	TTTTCACTAA
2915 2920	2925 2930	2935 2940	2945 2950	2955 2960	2965 2970
ATCTCTTTGA	TCTACTTGCC	TCATTTCCCT	ATCTTCTCCC	CCACGGTATC	CTAAACTTTA
TAGAGAAACT	AGATGAACGG	AGTAAAGGGA	TAGAAGAGGG	GGTGCCATAG	GATTTGAAAT
2975 2980	2985 2990	2995 3000	3005 3010	3015 3020	3025 3030
GACTTCCAC	TGTCTGAAA	GGAGACATTG	CTCTATGTCT	GCCTTCGACC	ACAGCAAGCC
CTGAAGGGTG	ACAAGACTTT	CCTCTGTAAC	GAGATACAGA	CGGAAGCTGG	TGTCGTTCCG
3035 3040	3045 3050	3055 3060	3065 3070	3075 3080	3085 3090
ATCATCTCTC	ATTGCTCCCG	GGGACTCAAG	AGGAATCTGT	TTCTCTGCTG	TCAACTTCCC
TAGTAGGAGG	TAACGAGGGC	CCCTGAGTTC	TCCTTAGACA	AAGAGACGAC	AGTTGAAGGG
3095 3100	3105 3110	3115 3120	3125 3130	3135 3140	3145 3150
ATCTGGCTCA	GCATAGGGTC	ACTTTGCCAT	TATGCAAATG	GAGATAAAAG	CAATTCTGGC
TAGACCGAGT	CGTATCCAG	TGAAACGGTA	ATACGTTTAC	CTCTATTTTC	GTTAAGACCG

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FIG. 6 - CONT'D

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3155 3160 3165 3170 3175 3180 3185 3190 3195 3200 3205 3210
*      *      *      *      *      *      *      *
TGTCCAGGAG CTAATCTGAC CGTTCCTATTG TGTGGATGAC CACATAAGAA GGCAATTTTA
ACAGGTCCTC GATTAGACTG GCAAGATAAC ACACCTACTG GTGTATTCTT CCGTTAAAT

3215 3220 3225 3230 3235 3240 3245 3250 3255 3260 3265 3270
*      *      *      *      *      *      *      *
GTGTATTAAT CATAGATTAT TATAAACTAT AAACCTTAAGG GCAAGGAGTT TATTACAATG
CACATAATTA GTATCTAATA ATATTTGATA TTTGAATTCC CGTTCCTCAA ATAATGTTAC

3275 3280 3285 3290 3295 3300 3305 3310 3315 3320 3325 3330
*      *      *      *      *      *      *      *
TATCTTTATT AAAACAAAAG GGTGTATAGT GTTCACAAAC TGTGAAAATA GTGTAAGAAC
ATAGAAATAA TTTTGTTC TCACATATCA CAAGTGTTTG ACACTTTAT CACATCTTG

3335 3340 3345 3350 3355 3360 3365 3370 3375 3380 3385 3390
*      *      *      *      *      *      *      *
TGTACATTGT GAGCTCTGGT TATTTTCTC TTGTACCATA GAAAAATGTA TAAAAATTAT
ACATGTAACA CTCGAGACCA ATAAAAAGAG AACATGGTAT CTTTTTACAT ATTTTAAATA

3395 3400 3405 3410 3415 3420 3425 3430 3435 3440 3445 3450
*      *      *      *      *      *      *      *
CAAAAAGCTA ATGTGCAGGG ATATTGCCTT ATTTGTCTGT AAAAAATGGA GCTCAGTAAC
GTTTTTCGAT TACACGTCCC TATAACGGAA TAAACAGACA TTTTTTACCT CGAGTCATTG

3455 3460 3465 3470 3475 3480 3485 3490 3495
*      *      *      *      *      *      *
ATAACTGCTT CTGGAGCTT TGGAATATTT TATCCTGTAT TCTTGTTT (SEQ ID NO:5)
TATTGACGAA GAACCTCGAA ACCTTATAAA ATAGGACATA AGAACAAA

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FIG. 7

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      5      10      15      20      25      30      35      40      45      50
      *      *      *      *      *      *      *      *      *      *
CAACA ATG GCG GCT CCG AGC CCG AGC GGT GGC GGC GGC AGC GGC ACC CCC
GTGTG TAC CGC CGA GGC TCG GGC TCG CCA CCG CCG CCG TCG CCG TGG GGG
Met Ala Ala Pro Ser Pro Ser Gly Gly Gly Gly Ser Gly Thr Pro>

      55      60      65      70      75      80      85      90      95
      *      *      *      *      *      *      *      *      *
GGC CCC GTA GGG TCC CCG GCG CCA GGC CAC CCG GCC GTC AGC AGC ATG
CCG GGG CAT CCC AGG GGC CGC GGT CCG GTG GGC CGG CAG TCG TCG TAC
Gly Pro Val Gly Ser Pro Ala Pro Gly His Pro Ala Val Ser Ser Met>

100    105    110    115    120    125    130    135    140    145
      *      *      *      *      *      *      *      *      *
CAG GGT AAA CGC AAA GCA CTG AAG TTG AAT TTT GCA AAT CCA CCT TTC
GTC CCA TTT GCG TTT CGT GAC TTC AAC TTA AAA CGT TTA GGT GGA AAG
Gln Gly Lys Arg Lys Ala Leu Lys Leu Asn Phe Ala Asn Pro Pro Phe>

      150    155    160    165    170    175    180    185    190
      *      *      *      *      *      *      *      *      *
AAA TCT ACA GCA AGG TTT ACT CTG AAT CCC AAT CCT ACA GGA GTT CAA
TTT AGA TGT CGT TCC AAA TGA GAC TTA GGG TTA GGA TGT CCT CAA GTT
Lys Ser Thr Ala Arg Phe Thr Leu Asn Pro Asn Pro Thr Gly Val Gln>

195    200    205    210    215    220    225    230    235    240
      *      *      *      *      *      *      *      *      *
AAC CCA CAC ATA GAG AGA CTG AGA ACA CAC AGC ATT GAG TCA TCA GGA
TTG GGT GTG TAT CTC TCT GAC TCT TGT GTG TCG TAA CTC AGT AGT CCT
Asn Pro His Ile Glu Arg Leu Arg Thr His Ser Ile Glu Ser Ser Gly>

245    250    255    260    265    270    275    280    285    290
      *      *      *      *      *      *      *      *      *
AAA CTG AAG ATC TCC CCT GAA CAA CAC TGG GAT TTC ACT GCA GAG GAC
TTT GAC TTC TAG AGG GGA CTT GTT GTG ACC CTA AAG TGA CGT CTC CTG
Lys Leu Lys Ile Ser Pro Glu Gln His Trp Asp Phe Thr Ala Glu Asp>

295    300    305    310    315    320    325    330    335
      *      *      *      *      *      *      *      *      *
TTG AAA GAC CTT GGA GAA ATT GGA CGA GGA GCT TAT GGT TCT GTC AAC
AAC TTT CTG GAA CCT CTT TAA CCT GCT CCT CGA ATA CCA AGA CAG TTG
Leu Lys Asp Leu Gly Glu Ile Gly Arg Gly Ala Tyr Gly Ser Val Asn>

340    345    350    355    360    365    370    375    380    385
      *      *      *      *      *      *      *      *      *
AAA ATG GTC CAC AAA CCA AGT GGG CAA ATA ATG GCA GTT AAA AGA ATT
TTT TAC CAG GTG TTT GGT TCA CCC GTT TAT TAC CGT CAA TTT TCT TAA
Lys Met Val His Lys Pro Ser Gly Gln Ile Met Ala Val Lys Arg Ile>

390    395    400    405    410    415    420    425    430
      *      *      *      *      *      *      *      *      *
CGG TCA ACA GTG GAT GAA AAA GAA CAA AAA CAA CTT CTT ATG GAT TTG
GCC AGT TGT CAC CTA CTT TTT CTT GTT TTT GTT GAA GAA TAC CTA AAC
Arg Ser Thr Val Asp Glu Lys Glu Gln Lys Gln Leu Leu Met Asp Leu>

435    440    445    450    455    460    465    470    475    480
      *      *      *      *      *      *      *      *      *
GAT GTA GTA ATG CCG AGT AGT GAT TGC CCA TAC ATT GTT CAG TTT TAT
CTA CAT CAT TAC GCC TCA TCA CTA ACG GGT ATG TAA CAA GTC AAA ATA
Asp Val Val Met Arg Ser Ser Asp Cys Pro Tyr Ile Val Gln Phe Tyr>

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FIG. 7 - CONT'D

485 490 495 500 505 510 515 520 525 530
 GGT GCA CTC TTC AGA GAG GGT GAC TGT TGG ATC TGT ATG GAA CTC ATG
 CCA CGT GAG AAG TCT CTC CCA CTG ACA ACC TAG ACA TAC CTT GAG TAC
 Gly Ala Leu Phe Arg Glu Gly Asp Cys Trp Ile Cys Met Glu Leu Met>

535 540 545 550 555 560 565 570 575
 TCT ACC TCG TTT GAT AAG TTT TAC AAA TAT GTA TAT AGT GTA TTA GAT
 AGA TGG AGC AAA CTA TTC AAA ATG TTT ATA CMT ATA TCA CAT AAT CTA
 Ser Thr Ser Phe Asp Lys Phe Tyr Lys Tyr Val Tyr Ser Val Leu Asp>

580 585 590 595 600 605 610 615 620 625
 GAT GTT ATT CCA GAA GAA ATT TTA GGC AAA ATC ACT TTA GCA ACT GTG
 CTA CAA TAA GGT CTT CTT TAA AAT CCG TTT TAG TGA AAT CGT TGA CAC
 Asp Val Ile Pro Glu Glu Ile Leu Gly Lys Ile Thr Leu Ala Thr Val>

630 635 640 645 650 655 660 665 670
 AAA GCA CTA AAC CAC TTA AAA GAA AAC TTG AAA ATT ATT CAC AGA GAT
 TTT CGT GAT TTG GTG AAT TTT CTT TTG AAC TTT TAA TAA GTG TCT CTA
 Lys Ala Leu Asn His Leu Lys Glu Asn Leu Lys Ile Ile His Arg Asp>

675 680 685 690 695 700 705 710 715 720
 ATC AAA CCT TCC AAT ATT CTT CTG GAC AGA AGT GGA AAT ATT AAG CTC
 TAG TTT GGA AGG TTA TAA GAA GAC CTG TCT TCA CCT TTA TAA TTC GAG
 Ile Lys Pro Ser Asn Ile Leu Leu Asp Arg Ser Gly Asn Ile Lys Leu>

725 730 735 740 745 750 755 760 765 770
 TGT GAC TTC GGC ATC AGT GGA CAG CTT GTG GAC TCT ATT GCC AAG ACA
 ACT CTG AAG CCG TAG TCA CCT GTC GAA CAC CTG AGA TAA CCG TTC TGT
 Cys Asp Phe Gly Ile Ser Gly Gln Leu Val Asp Ser Ile Ala Lys Thr>

775 780 785 790 795 800 805 810 815
 AGA GAT GCT GGC TGT AGG CCA TAC ATG GCA CCT GAA AGA ATA GAC CCA
 TCT CTA CGA CCG ACA TCC GGT ATG TAC CGT GGA CTT TCT TAT CTG GGT
 Arg Asp Ala Gly Cys Arg Pro Tyr Met Ala Pro Glu Arg Ile Asp Pro>

820 825 830 835 840 845 850 855 860 865
 AGC GCA TCA CGA CAA GGA TAT GAT GTC CGC TCT GAT GTC TGG AGT TTG
 TCG CGT AGT GCT GTT CCT ATA CTA CAG GCG AGA CTA CAG ACC TCA AAC
 Ser Ala Ser Arg Gln Gly Tyr Asp Val Arg Ser Asp Val Trp Ser Leu>

870 875 880 885 890 895 900 905 910
 GGG ATC ACA TTG TAT GAG TTG GCC ACA GGC CGA TTT CCT TAT CCA AAG
 CCC TAG TGT AAC ATA CTC AAC CGG TGT CCG GCT AAA GGA ATA GGT TTC
 Gly Ile Thr Leu Tyr Glu Leu Ala Thr Gly Arg Phe Pro Tyr Pro Lys>

915 920 925 930 935 940 945 950 955 960
 TGG AAT AGT GTA TTT GAT CAA CTA ACA CAA GTC GTG AAA GGA GAT CCT
 ACC TTA TCA CAT AAA CTA GTT GAT TGT GTT CAG CAC TTT CCT CTA GGA
 Trp Asn Ser Val Ph Asp Gln Leu Thr Gln Val Val Lys Gly Asp Pro>

965 970 975 980 985 990 995 1000 1005 1010

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FIG. 7 - CONT'D

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CCG CAG CTG AGT AAT TCT GAG GAA AGG GAA TTC TCC CCG AGT TTC ATC
GGC GTC GAC TCA TTA AGA CTC CTT TCC CTT AAG AGG GGC TCA AAG TAG
Pro Gln Leu Ser Asn Ser Glu Glu Arg Glu Phe Ser Pro Ser Phe Ile>

1015 1020 1025 1030 1035 1040 1045 1050 1055
AAC TTT GTC AAC TTG TGC CTT ACG AAG GAT GAA TCC AAA AGG CCA AAG
TTG AAA CAG TTG AAC ACG GAA TGC TTC CTA CTT AGG TTT TCC GGT TTC
Asn Phe Val Asn Leu Cys Leu Thr Lys Asp Glu Ser Lys Arg Pro Lys>

1060 1065 1070 1075 1080 1085 1090 1095 1100 1105
TAT AAA GAG CTT CTG AAA CAT CCC TTT ATT TTG ATG TAT GAA GAA CGT
ATA TTT CTC GAA GAC TTT GTA GGG AAA TAA AAC TAC ATA CTT CTT GCA
Tyr Lys Glu Leu Leu Lys His Pro Phe Ile Leu Met Tyr Glu Glu Arg>

1110 1115 1120 1125 1130 1135 1140 1145 1150
GCC GTT GAG GTC GCA TGC TAT GTT TGT AAA ATC CTG GAT CAA ATG CCA
CGG CAA CTC CAG CGT ACG ATA CAA ACA TTT TAG GAC CTA GTT TAC GGT
Ala Val Glu Val Ala Cys Tyr Val Cys Lys Ile Leu Asp Gln Met Pro>

1155 1160 1165 1170 1175 1180 1185 1190 1195 1200
GCT ACT CCC AGC TCT CCC ATG TAT GTC GAT TGATAT CGTGTCTACA
CGA TGA GGG TCG AGA GGG TAC ATA CAG CTA ACTATA GCRACGATGT
Ala Thr Pro Ser Ser Pro Met Tyr Val Asp> (SEQ ID NO:8)

1205 1210 1215 1220 1225 1230 1235 1240 1245 1250 1255 1260
TCAGACTCTA GAAAAAAGGG CTGAGAGGAA GCAAGACGTA AAGAATTTTC ATCCCGTATC
AGTCTGAGAT CTTTTTTTCCC GACTCTCCTT CGTTCTGCAT TTCTTAAAAG TAGGGCATAG

1265 1270 1275 1280 1285 1290 1295 1300 1305 1310 1315 1320
ACAGTGTTTT TATTGCTCGC CCAGACACCA TGTGCAATAA GATTGGTGTT CGTTTCCATC
TGTCAAAAAA ATAACGAGCG GGTCTGTGGT ACACGTTATT CTAACCACAA GCAAAGGTAG

1325 1330 1335 1340 1345 1350 1355 1360 1365 1370 1375 1380
ATGTCTGTAT ACTCCTGTCA CCTAGAACGT GCATCCTTGT AATACCTGAT TGATCACACA
TACAGACATA TGAGGACAGT GGATCTTGCA CGTAGGAACA TTATGGACTA ACTAGTGTGT

1385 1390 1395 1400 1405 1410 1415 1420 1425 1430 1435 1440
GTGTTAGTGC TGGTCAGAGA GACCTCATCC TGCTCTTTTG TGATGAACAT ATTCATGAAA
CACAATCAG ACCAGTCTCT CTGGAGTAGG ACGAGAAAAC ACTACTTGTA TAAGTACTTT

1445 1450 1455 1460 1465 1470 1475 1480 1485 1490 1495 1500
TGTGGAAGTC AGTACGATCA AGTTGTTGAC TGTGATTAGA TCACATCTTA AATTCATTTC
ACACCTTCAG TCATGCTAGT TCAACAACTG ACACATACTT AGTGTAAGT TTAAGTAAAG

1505 1510 1515 1520 1525 1530 1535 1540 1545 1550 1555 1560
TAGACTCAAA ACCTGGAGAT GCAGCTACTG GAATGGTGTT TTGTCAGACT TCCAAATCCT
ATCTGAGTTT TGGACCTCTA CGTCGATGAC CTTACCACAA AACAGTCTGA AGGTTTAGGA

1565 1570 1575 1580 1585 1590 1595 1600 1605 1610 1615 1620
GGAAGGACAC AGTGATGAAT GTACTATATC TGAACATAGA AACTCGGGCT TGAGTGAGAA
CCTTCTGTG TCACTACTTA CATGATATAG ACTTGATCTT TTGAGCCCGA ACTCACTCTT

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FIG. 7 - CONT'D

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1625 1630 1635 1640 1645 1650 1655 1660 1665 1670 1675 1680
GAGCTTGCAC AGCCAACGAG ACACATTGCC TTCTGGAGCT GGGAGACAAA GGAGGAATTT
CTCGAACGTG TCGGTTGCTC TGTGTAACGG AAGACCTCGA CCTCTGTTT CCTCCTTAAA

1685 1690 1695 1700 1705 1710 1715 1720 1725 1730 1735 1740
ACTTTCTTCA CCAAGTGCAA TAGATTACTG ATGTGATATT CTGTTGCTTT ACAGTTACAG
TGAAAGAAGT GGTTCACGTT ATCTAATGAC TACACTATAA GACAACGAAA TGTCAATGTC

1745 1750 1755 1760 1765 1770 1775 1780 1785 1790 1795 1800
TTGATGTTTG GGGATCGATG TGCTCAGCCA AATTTCTCTG TTGAAATATC ATGTTAAATT
AACTACAAAC CCTAGCTAC ACCAGTCGGT TTAAAGGACA AACTTTATAG TACAATTTAA

1805 1810 1815 1820 1825 1830 1835 1840 1845 1850 1855 1860
AGAATGAATT TATCTTTACC AAAAACCATG TTGCGTTCAA AGAGGTGAAC ATTAAAATAT
TCTTACTTAA ATAGAAATGG TTTTGGTAC AACGCAAGTT TCTCCACTTG TAATTTTATA

1865 1870 1875 1880 1885 1890 1895 1900 1905 1910 1915 1920
AGAGACAGGA CAGAATGTGT TCTTTTCTCC TCTACCAGTC CTATTTTTC A TGGAAGAC
TCTCTGTCCT GTCTTACACA AGAAAAGAGG AGATGGTCAG GATAAAAAGT TACCCTCTG

1925 1930 1935 1940 1945 1950 1955 1960 1965 1970 1975 1980
TCAGGAGTCT GCCACTTGTG AAAGAAGGTG CTGATCCTAA GAATTTTTC TTCTCAGAAT
AGTCCTCAGA CCGTGAACAG TTTCTTCCAC GACTAGGATT CTTAAAAAGT AAGAGTCTTA

1985 1990 1995 2000 2005 2010 2015 2020 2025 2030 2035 2040
TCGGTGTGCT GCCAACTTGA TGTTCACCT GCCACAAACC ACCAGGACTG AAAGAAGAAA
AGCCACACGA CCGTTGAACT ACAAGGTGGA CCGTGTGTTG TGGTCTGAC TTTCTTCTTT

2045 2050 2055 2060 2065 2070 2075 2080 2085 2090 2095 2100
ACAGTACAGA AGGCAAAGTT TACAGATGTT TTTAATTCTA GTATTTTATC TGGAAACAT
TGTATGTCT TCCGTTTCAA ATGTCTACAA AAATTAAGAT CATAAATAG ACCTTGTGTA

2105 2110 2115 2120 2125 2130 2135 2140 2145 2150 2155 2160
TGTAGCAGCT ATATATTTC CCTTGGTCCC AAGCCTGATA CTTTAGCCAT CATAACTCAC
ACATCGTCGA TATATAAAGG GGAACCAGGG TTCGGACTAT GAAATCGGTA GTATTGAGTG

2165 2170 2175 2180 2185 2190 2195 2200 2205 2210 2215 2220
TAACAGGGAG AAGTAGCTAG TAGCAATGTG CCTTGATTGA TTAGATAAAG ATTCTAGTA
ATTGTCCCTC TTCATCGATC ATCGTTACAC GGAACCTAAT AATCTATTTC TAAAGATCAT

2225 2230 2235 2240 2245 2250 2255 2260 2265 2270 2275 2280
GGCAGCAAAA GACCAAATCT CAGTTGTTTG CTTCTTGCCA TCACTGGTCC AGGTCTTCAG
CCGTCGTTTT CTGGTTTAGA GTCAACAAAC GAAGAACGGT AGTGACCAGG TCCAGAAGTC

2285 2290 2295 2300 2305 2310 2315 2320 2325 2330 2335 2340
TTTCCGAATC TCTTTCCTTT CCCTGTGGT CTATTGTGCG TATGTGACTT GCGCTTAATC
AAAGGCTTAG AGAAAGGGAA GGGGACACCA GATAACAGCG ATACACTGAA CGGAATTAG

2345 2350 2355 2360 2365 2370 2375 2380 2385 2390 2395 2400

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FIG. 7 - CONT'D

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      *           *           *           *           *
CAATATTTTG CCTTTTTTCT ATATCAAAAA ACCTTTACAG TTAGCAGGGA TGTTCCTTAC
GTTATAAAAC GGAAAAAGA TATAGTTTTT TGGAAATGTC AATCGTCCCT ACAAGGAATG

2405 2410 2415 2420 2425 2430 2435 2440 2445 2450 2455 2460
      *           *           *           *           *
CGAGGATTTT TAACCCCCAA TCTCTCATAA TCGCTAGTGT TTAAAAGGCT AAGAATAGTG
GCTCCTAAAA ATTGGGGGTT AGAGAGTATT AGCGATCACA AATTTTCCGA TTCTTATCAC

2465 2470 2475 2480 2485 2490 2495 2500 2505 2510 2515 2520
      *           *           *           *           *
GGGCCCCAACC GATGTGGTAG GTGATAAAGA GGCATCTTTT CTAGAGACAC ATTGGACCAG
CCCGGGTTGG CTACACCATC CACTATTTCT CCGTAGAAAA GATCTCTGTG TAACCTGGTC

2525 2530 2535 2540 2545 2550 2555 2560 2565 2570 2575 2580
      *           *           *           *           *
ATGAGGATCC GAAACGGCAG CCTTTACGTT CATCACCTGC TAGAACCTCT CGTAGTCCAT
TACTCCTAGG CTTTGCCGTC GGAAATGCAA GTAGTGGACG ATCTTGGAGA GCATCAGGTA

2585 2590 2595 2600 2605 2610 2615 2620 2625 2630 2635 2640
      *           *           *           *           *
CACCATTTCT TGGCATTGGA ATTCTACTGG AAAAAAATAC AAAAAGCAAA ACAAACCCCT
GTGGTAAAGA ACCGTAACTT TAAGATGACC TTTTTTTATG TTTTTCGTTT TGTTTTGGGA

2645 2650 2655 2660 2665 2670 2675 2680 2685 2690 2695 2700
      *           *           *           *           *
CAGCACTGTT ACAAGAGGCC ATTTAAGTAT CTGTGCTTC TTCACTTACC CATTAGCCAG
GTCGTGACAA TGTTCCTCCG TAAATTCATA GAACACGAAG AAGTGAATGG GTAATCGGTC

2705 2710 2715 2720 2725 2730 2735 2740 2745 2750 2755 2760
      *           *           *           *           *
GTTCTCATTA GGTTTTGCTT GGGCCTCCCT GGCACGTAAC CTTAGGCTTT GTATGACAGT
CAAGAGTAAT CCAAAACGAA CCCCGAGGGA COGTGACTTG GAATCCGAAA CATACTGTCA

2765 2770 2775 2780 2785 2790 2795 2800 2805 2810 2815 2820
      *           *           *           *           *
GAAGCAGCAC TGTGAGTGGT TCAAGCACAC TGGAATATAA AACAGTCATG GCCTGAGATG
CTTCGTCGTG AACTCACCA AGTTCGTGTG ACCTTATATT TTGTCAGTAC CGACTCTAC

2825 2830 2835 2840 2845 2850 2855 2860 2865 2870 2875 2880
      *           *           *           *           *
CAGGTGATGC CATTACAGAA CCAAATCGTG GCACGTATTG CTGTGTCTCC TCTCAGAGTG
GTCCACTACG GTAATGTCTT GGTTTAGCAC CGTGCATAAC GACACAGAGG AGAGTCTCAC

2885 2890 2895 2900 2905 2910 2915 2920 2925 2930 2935 2940
      *           *           *           *           *
ACAGTCATAA ATACTGTCAA ACAATAAAGG GAGAATGGTG CTGTTTAAAG TCACATCCCT
TGTCAGTATT TATGACAGTT TGTATTTCCT CTCTTACCAC GACAAATTTC AGTGTAGGGA

2945 2950 2955 2960 2965 2970 2975 2980 2985 2990 2995 3000
      *           *           *           *           *
GTAAATTGCA GAATTCAAAA GTGATTATCT CTTTGATCTA CTTGCCTCAT TTCCCTATCT
TATTTAACGT CTTAAGTTTT CACTAATAGA GAAACTAGAT GAACGGAGTA AAGGGATAGA

3005 3010 3015 3020 3025 3030 3035 3040 3045 3050 3055 3060
      *           *           *           *           *
TCTCCCCCAC GGTATCCTAA ACTTTAGACT TCCCACTGTT CTGAAAGGAG ACATTGCTCT
AGAGGGGGTG CCATAGGATT TGAAATCTGA AGGGTGACAA GACTTTCCTC TGTAACGAGA

3065 3070 3075 3080 3085 3090 3095 3100 3105 3110 3115 3120
      *           *           *           *           *
ATGTCCTGCCT TCGACCACAG CAAGCCATCA TCCTCCATTG CTCCCGGGGA CTCAAGAGGA

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FIG. 7 - CONT'D

TACAGACGGA AGCTGGTGTC GTTCGGTAGT AGGAGGTAAC GAGGGCCCCCT GAGTTCTCCT
3125 3130 3135 3140 3145 3150 3155 3160 3165 3170 3175 3180
ATCTGTTTCT CTGCTGTCAA CTTCCCATCT GGCTCAGCAT AGGGTCACTT TGCCATTATG
TAGACAAAGA GACGACAGTT GAAGGGTAGA CCGAGTCGTA TCCCAGTGAA ACGGTAATAC
3185 3190 3195 3200 3205 3210 3215 3220 3225 3230 3235 3240
CAAATGGAGA TAAAAGCAAT TCTGGCTGTC CAGGAGCTAA TCTGACCGTT CTATTGTGTG
GTTTACCTCT ATTTTCGTTA AGACCGACAG GTCCTCGATT AGACTGGCAA GATAACACAC
3245 3250 3255 3260 3265 3270 3275 3280 3285 3290 3295 3300
GATGACCACA TAAGAAGGCA ATTTTAGTGT ATTAATCATA GATTATTATA AACTATAAAC
CTACTGGTGT ATTCTTCCGT TAAAATCACA TAATTAGTAT CTAATAATAT TTGATATTG
3305 3310 3315 3320 3325 3330 3335 3340 3345 3350 3355 3360
TTAAGGGCAA GGAGTTTATT ACAATGTATC TTTATTAAAA CAAAAGGGTG TATAGTGTTC
AATTCCCGTT CCTCAAATAA TGTTACATAG AAATAATTTT GTTTTCCAC ATATCACAAG
3365 3370 3375 3380 3385 3390 3395 3400 3405 3410 3415 3420
ACAACTGTG AAAATAGTGT AAGAACTGTA CATTGTGAGC TCTGGTTATT TTTCTCTTGT
TGTTTGACAC TTTTATCACA TTCTTGACAT GTAACACTCG AGACCAATAA AAAGAGAACA
3425 3430 3435 3440 3445 3450 3455 3460 3465 3470 3475 3480
ACCATAGAAA AATGTATAAA AATTATCAAA AAGCTAATGT GCAGGGATAT TGCCTTATTT
TGGTATCTTT TTACATATTT TTAATAGTTT TTCGATTACA CGTCCCTATA ACGGAATAAA
3485 3490 3495 3500 3505 3510 3515 3520 3525 3530 3535 3540
GTCTGTAAAA AATGGAGCTC AGTAACATAA CTGCTTCTTG GAGCTTTGGA ATATTTTATC
CAGACATTTT TTACCTCGAG TCATTGTATT GACGAAGAAC CTCGAAACCT TATAAAATAG
3545 3550
CTGTATTCTT GTTT (SEQ ID NO:7)
GACATAAGAA CAAA

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FIG. 8

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      5      10      15      20      25      30      35      40      45      50
      *      *      *      *      *      *      *      *      *      *
CTCCCAACA ATG GCG GCT CCG AGC CCG AGC GGC GGC GGC GGC TCC GGC GGC
GAGGGTTGT TAC CGC CGA GGC TCG GGC TCG CCG CCG CCG CCG AGG CCC CCG
Met Ala Ala Pro Ser Pro Ser Gly Gly Gly Gly Ser Gly Gly>

      55      60      65      70      75      80      85      90      95
      *      *      *      *      *      *      *      *      *
GGC AGC GGC AGC GGC ACC CCC GGC CCC GTA GGC TCC CCG GCG CCA GGC
CCG TCG CCG TCG CCG TGG GGG CCG GGG CAT CCC AGG GGC CCG GGT CCG
Gly Ser Gly Ser Gly Thr Pro Gly Pro Val Gly Ser Pro Ala Pro Gly>

100    105    110    115    120    125    130    135    140    145
      *      *      *      *      *      *      *      *      *      *
CAC CCG GCC GTC AGC AGC ATG CAG GGT AAA CGC AAA GCA CTG AAG TTG
GTG GGC CGG CAG TCG TCG TAC GTC CCA TTT GCG TTT CGT GAC TTC AAC
His Pro Ala Val Ser Ser Met Gln Gly Lys Arg Lys Ala Leu Lys Leu>

150    155    160    165    170    175    180    185    190    195
      *      *      *      *      *      *      *      *      *      *
AAT TTT GCA AAT CCA CCT TTC AAA TCT ACA GCA AGG TTT ACT CTG AAT
TTA AAA CGT TTA GGT GGA AAG TTT AGA TGT CGT TCC AAA TGA GAC TTA
Asn Phe Ala Asn Pro Pro Phe Lys Ser Thr Ala Arg Phe Thr Leu Asn>

200    205    210    215    220    225    230    235    240
      *      *      *      *      *      *      *      *      *
CCC AAT CCT ACA GGA GTT CAA AAC CCA CAC ATA GAG AGA CTG AGA ACA
GGG TTA GGA TGT CCT CAA GTT TTG GGT GTG TAT CTC TCT GAC TCT TGT
Pro Asn Pro Thr Gly Val Gln Asn Pro His Ile Glu Arg Leu Arg Thr>

245    250    255    260    265    270    275    280    285    290
      *      *      *      *      *      *      *      *      *      *
CAC AGC ATT GAG TCA TCA GGA AAA CTG AAG ATC TCC CCT GAA CAA CAC
GTG TCG TAA CTC AGT AGT CCT TTT GAC TTC TAG AGG GGA CTT GTT GTG
His Ser Ile Glu Ser Ser Gly Lys Leu Lys Ile Ser Pro Glu Gln His>

295    300    305    310    315    320    325    330    335
      *      *      *      *      *      *      *      *      *
TGG GAT TTC ACT GCA GAG GAC TTG AAA GAC CTT GGA GAA ATT GGA CGA
ACC CTA AAG TGA CGT CTC CTG AAC TTT CTG GAA CCT CTT TAA CCT GCT
Trp Asp Phe Thr Ala Glu Asp Leu Lys Asp Leu Gly Glu Ile Gly Arg>

340    345    350    355    360    365    370    375    380    385
      *      *      *      *      *      *      *      *      *      *
GGA GCT TAT GGT TCT GTC AAC AAA ATG GTC CAC AAA CCA AGT GGC CAA
CCT CGA ATA CCA AGA CAG TTG TTT TAC CAG GTG TTT GGT TCA CCC GTT
Gly Ala Tyr Gly Ser Val Asn Lys Met Val His Lys Pro Ser Gly Gln>

390    395    400    405    410    415    420    425    430    435
      *      *      *      *      *      *      *      *      *      *
ATA ATG GCA GTT AAA AGA ATT CGG TCA ACA GTG GAT GAA AAA GAA CAA
TAT TAC CGT CAA TTT TCT TAA GCC AGT TGT CAC CTA CTT TTT CTT GTT
Ile Met Ala Val Lys Arg Ile Arg Ser Thr Val Asp Glu Lys Glu Gln>

440    445    450    455    460    465    470    475    480
      *      *      *      *      *      *      *      *      *
AAA CAA CTT CTT ATG GAT TTG GAT GTA GTA ATG CGG AGT AGT GAT TGC
TTT GTT GAA GAA TAC CTA AAC CTA CAT CAT TAC GCC TCA TCA CTA ACG
Lys Gln Leu Leu Met Asp Leu Asp Val Val Met Arg Ser Ser Asp Cys>

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FIG. 8 - CONT'D

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485      490      495      500      505      510      515      520      525      530
      *      *      *      *      *      *      *      *      *
CCA TAC ATT GTT CAG TTT TAT GGT GCA CTC TTC AGA GAG GGT GAC TGT
GGT ATG TAA CAA GTC AAA ATA CCA CGT GAG AAG TCT CTC CCA CTG ACA
Pro Tyr Ile Val Gln Phe Tyr Gly Ala Leu Phe Arg Glu Gly Asp Cys>

535      540      545      550      555      560      565      570      575
      *      *      *      *      *      *      *      *
TGG ATC TGT ATG GAA CTC ATG TCT ACC TCG TTT GAT AAG TTT TAC AAA
ACC TAG ACA TAC CTT GAG TAC AGA TGG AGC AAA CTA TTC AAA ATG TTT
Trp Ile Cys Met Glu Leu Met Ser Thr Ser Phe Asp Lys Phe Tyr Lys>

580      585      590      595      600      605      610      615      620      625
      *      *      *      *      *      *      *      *
TAT GTA TAT AGT GTA TTA GAT GAT GTT ATT CCA GAA GAA ATT TTA GGC
ATA CAT ATA TCA CAT AAT CTA CTA CAA TAA GGT CTT CTT TAA AAT CCG
Tyr Val Tyr Ser Val Leu Asp Asp Val Ile Pro Glu Glu Ile Leu Gly>

630      635      640      645      650      655      660      665      670      675
      *      *      *      *      *      *      *
AAA ATC ACT TTA GCA ACT GTG AAA GCA CTA AAC CAC TTA AAA GAA AAC
TTT TAG TGA AAT CGT TGA CAC TTT CGT GAT TTG GTG AAT TTT CTT TTG
Lys Ile Thr Leu Ala Thr Val Lys Ala Leu Asn His Leu Lys Glu Asn>

680      685      690      695      700      705      710      715      720
      *      *      *      *      *      *      *
TTG AAA ATT ATT CAC AGA GAT ATC AAA CCT TCC AAT ATT CTT CTG GAC
AAC TTT TAA TAA GTG TCT CTA TAG TTT GGA AGG TTA TAA GAA GAC CTG
Leu Lys Ile Ile His Arg Asp Ile Lys Pro Ser Asn Ile Leu Leu Asp>

725      730      735      740      745      750      755      760      765      770
      *      *      *      *      *      *      *
AGA AGT GGA AAT ATT AAG CTC TGT GAC TTC GGC ATC AGT GGA CAG CTT
TCT TCA CCT TTA TAA TTC GAG ACA CTG AAG CCG TAG TCA CCT GTC GAA
Arg Ser Gly Asn Ile Lys Leu Cys Asp Phe Gly Ile Ser Gly Gln Leu>

775      780      785      790      795      800      805      810      815
      *      *      *      *      *      *      *
GTG GAC TCT ATT GCC AAG ACA AGA GAT GCT GGC TGT AGG CCA TAC ATG
CAC CTG AGA TAA CGG TTC TGT TCT CTA CGA CCG ACA TCC GGT ATG TAC
Val Asp Ser Ile Ala Lys Thr Arg Asp Ala Gly Cys Arg Pro Tyr Met>

820      825      830      835      840      845      850      855      860      865
      *      *      *      *      *      *      *
GCA CCT GAA AGA ATA GAC CCA AGC GCA TCA CGA CAA GGA TAT GAT GTC
CGT GGA CTT TCT TAT CTG GGT TCG CGT AGT GCT GTT CCT ATA CTA CAG
Ala Pro Glu Arg Ile Asp Pro Ser Ala Ser Arg Gln Gly Tyr Asp Val>

870      875      880      885      890      895      900      905      910      915
      *      *      *      *      *      *      *
CGC TCT GAT GTC TGG AGT TTG GGG ATC ACA TTG TAT GAG TTG GCC ACA
GCG AGA CTA CAG ACC TCA AAC CCC TAG TGT AAC ATA CTC AAC CGG TGT
Arg Ser Asp Val Trp Ser Leu Gly Ile Thr Leu Tyr Glu Leu Ala Thr>

920      925      930      935      940      945      950      955      960
      *      *      *      *      *      *      *
GGC CGA TTT CCT TAT CCA AAG TGG AAT AGT GTA TTT GAT CAA CTA ACA
CCG GCT AAA GGA ATA GGT TTC ACC TTA TCA CAT AAA CTA GTT GAT TGT
Gly Arg Phe Pro Tyr Pro Lys Trp Asn Ser Val Phe Asp Gln Leu Thr>

965      970      975      980      985      990      995      1000      1005      1010
      *      *      *      *      *      *      *

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FIG. 8 - CONT'D

CAA GTC GTG AAA GGA GAT CCT CCG CAG CTG AGT AAT TCT GAG GAA AGG
 GTT CAG CAC TTT CCT CTA GGA GGC GTC GAC TCA TTA AGA CTC CTT TCC
 Gln Val Val Lys Gly Asp Pro Pro Gln Leu Ser Asn Ser Glu Glu Arg>
 1015 1020 1025 1030 1035 1040 1045 1050 1055
 GAA TTC TCC CCG AGT TTC ATC AAC TTT GTC AAC TTG TGC CTT ACG AAG
 CTT AAG AGG GGC TCA AAG TAG TTG AAA CAG TTG AAC ACG GAA TGC TTC
 Glu Phe Ser Pro Ser Phe Ile Asn Phe Val Asn Leu Cys Leu Thr Lys>
 1060 1065 1070 1075 1080 1085 1090 1095 1100 1105
 GAT GAA TCC AAA AGG CCA AAG TAT AAA GAG CTT CTG AAA CAT CCC TTT
 CTA CTT AGG TTT TCC GGT TTC ATA TTT CTC GAA GAC TTT GTA GGG AAA
 Asp Glu Ser Lys Arg Pro Lys Tyr Lys Glu Leu Leu Lys His Pro Phe>
 1110 1115 1120 1125 1130 1135 1140 1145 1150 1155
 ATT TTG ATG TAT GAA GAA CGT GCC GTT GAG GTC GCA TGC TAT GTT TGT
 TAA AAC TAC ATA CTT CTT GCA CGG CAA CTC CAG CGT ACG ATA CAA ACA
 Ile Leu Met Tyr Glu Glu Arg Ala Val Glu Val Ala Cys Tyr Val Cys>
 1160 1165 1170 1175 1180 1185 1190 1195 1200
 AAA ATC CTG GAT CAA ATG CCA GCT ACT CCC AGC TCT CCC ATG TAT GTC
 TTT TAG GAC CTA GTT TAC GGT CGA TGA GGG TCG AGA GGG TAC ATA CAG
 Lys Ile Leu Asp Gln Met Pro Ala Thr Pro Ser Ser Pro Met Tyr Val>
 1205 1210 1215 1220 1225 1230 1235 1240 1245 1250 1255 1260
 GAT TGAT ATCGCTGCTA CATCAGACTC TAGAAAAAAG GGCTGAGAGG AAGCAAGACG
 CTA ACTA TAGCGACGAT GTAGTCTGAG ATCTTTTTTC CCGACTCTCC TTCGTTCTGC
 Asp> (SEQ ID NO:10)
 1265 1270 1275 1280 1285 1290 1295 1300 1305 1310 1315 1320
 TAAAGAATTT TCATCCCGTA TCACAGTGT TTTATTGCTC GCCCAGACAC CATGTGCAAT
 ATTTCTTAAA AGTAGGGCAT AGTGTCACAA AAATAACGAG CGGGTCTGTG GTACACGTTA
 1325 1330 1335 1340 1345 1350 1355 1360 1365 1370 1375 1380
 AAGATTGGTG TTCGTTTCCA TCATGTCTGT ATACTCCTGT CACCTAGAAC GTGCATCCTT
 TTCTAACCAC AAGCAAAGGT AGTACAGACA TATGAGGACA GTGGATCTTG CACGTAGGAA
 1385 1390 1395 1400 1405 1410 1415 1420 1425 1430 1435 1440
 GTAATACCTG ATTGATCACA CAGTGTTAGT GCTGGTCAGA GAGACCTCAT CCTGCTCTTT
 CATTATGGAC TAACTAGTGT GTCACAATCA CGACCAGTCT CTCTGGAGTA GGACGAGAAA
 1445 1450 1455 1460 1465 1470 1475 1480 1485 1490 1495 1500
 TGTGATGAAC ATATTCATGA AATGTGGAAG TCAGTACGAT CAAGTTGTTG ACTGTGATTA
 ACACTACTTG TATAAGTACT TTACACCTTC AGTCATGCTA GTTCAACAAC TGACACTAAT
 1505 1510 1515 1520 1525 1530 1535 1540 1545 1550 1555 1560
 GATCACATCT TAAATTCATT TCTAGACTCA AAACCTGGAG ATGCAGCTAC TGGAAATGGTG
 CTAGTGTAGA ATTTAAGTAA AGATCTGAGT TTTGGACCTC TACGTCGATG ACCTTACCAC
 1565 1570 1575 1580 1585 1590 1595 1600 1605 1610 1615 1620
 TTTTGTGAGA CTTCCAAATC CTGGAAGGAC ACAGTGATGA ATGTACTATA TCTGAACATA

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FIG. 8 - CONT'D

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AAAACAGTCT GAAGGTTTAG GACCTTCCTG TGTCACTACT TACATGATAT AGACTTGTAT
1625 1630 1635 1640 1645 1650 1655 1660 1665 1670 1675 1680
GAAACTCGGG CTTGAGTGAG AAGAGCTTGC ACAGCCAACG AGACACATTG CCTTCTGGAG
CTTTGAGCCC GAACTCACTC TTCTCGAACG TGTCGGTTGC TCTGTGTAAC GGAAGACCTC
1685 1690 1695 1700 1705 1710 1715 1720 1725 1730 1735 1740
CTGGGAGACA AAGGAGGAAT TTACTTTCTT CACCAAGTGC AATAGATTAC TGATGTGATA
GACCTCTGTG TTCTCCTTA AATGAAAGAA GTGGTTCACG TTATCTAATG ACTACACTAT
1745 1750 1755 1760 1765 1770 1775 1780 1785 1790 1795 1800
TTCTGTTGCT TTACAGTTAC AGTTGATGTT TGGGGATCGA TGTGCTCAGC CAAATTTCTT
AAGACAACGA AATGTCAATG TCAACTACAA ACCCCTAGCT ACACGAGTCG GTTTAAAGGA
1805 1810 1815 1820 1825 1830 1835 1840 1845 1850 1855 1860
GTTTGAAATA TCATGTTAAA TTAGAATGAA TTTATCTTTA CCAAAAACCA TGTTCGTTTC
CAAACCTTAT AGTACAATTT AATCTTACTT AAATAGAAAT GGTPTTGGT ACAACGCAAG
1865 1870 1875 1880 1885 1890 1895 1900 1905 1910 1915 1920
AAAGAGGTGA ACATTAAAAT ATAGAGACAG GACAGAATGT GTTCTTTTCT CCTCTACCAG
TTTCTCCACT TGTAAATTTA TATCTCTGTC CTGTCTTACA CAAGAAAAGA GGAGATGGTC
1925 1930 1935 1940 1945 1950 1955 1960 1965 1970 1975 1980
TCCTATTTTT CAATGGGAAG ACTCAGGAGT CTGCCACTTG TCAAAGAAGG TGCTGATCCT
AGGATAAAAA GTTACCCTTC TGAGTCCTCA GACGGTGAAC AGTTTCTTCC ACGACTAGGA
1985 1990 1995 2000 2005 2010 2015 2020 2025 2030 2035 2040
AAGAATTTTT CATTCTCAGA ATTCCGTGTG CTGCCAACTT GATGTTCCAC CTGCCACAAA
TTCTTAAAAA GTAAGAGTCT TAAGCCACAC GACGGTTGAA CTACAAGGTG GACGGTGTTC
2045 2050 2055 2060 2065 2070 2075 2080 2085 2090 2095 2100
CCACCAGGAC TGAAAGAAGA AAACAGTACA GAAGGCAAAG TTTACAGATG TTTTAAATTC
GGTGGTCTCG ACTTCTCTCT TTTGTGATGT CTTCGGTTTC AAATGTCTAC AAAAATTAAG
2105 2110 2115 2120 2125 2130 2135 2140 2145 2150 2155 2160
TAGTATTTTA TCTGGAACAA CTTGTAGCAG CTATATATTT CCCCTTGGTC CCAAGCCTGA
ATCATAAAAT AGACCTTGTT GAACATCGTC GATATATAAA GGGGAACCAG GGTTCGGACT
2165 2170 2175 2180 2185 2190 2195 2200 2205 2210 2215 2220
TACTTTAGCC ATCATAACTC ACTAACAGGG AGAAGTAGCT AGTAGCAATG TGCTTGTATT
ATGAAATCGG TAGTATTGAG TGATTGTCCC TCTTCATCGA TCATCGTTAC ACGGAACATA
2225 2230 2235 2240 2245 2250 2255 2260 2265 2270 2275 2280
GATTAGATAA AGATTTCTAG TAGGCAGCAA AAGACCAAAT CTCAGTTGTT TGCTTCTTGC
CTAATCTATT TCTAAAGATC ATCCGTCGTT TTCTGGTTTA GAGTCAACAA ACGAAGAACG
2285 2290 2295 2300 2305 2310 2315 2320 2325 2330 2335 2340
CATCACTGGT CCAGGTCTTC AGTTTCCGAA TCTCTTTCCC TTCCCCTGTG GTCTATTGTC
GTAGTGACCA GGTCCAGAAG TCAAAGGCTT AGAGAAAGGG AAGGGGACAC CAGATAACAG

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FIG. 8 - CONT'D

2345	2350	2355	2360	2365	2370	2375	2380	2385	2390	2395	2400
GCTATGTGAC	TTGCGCTTAA	TCCAATATTT	TGCCTTTTTT	CTATATCAAA	AAACCTTTAC						
CGATACACTG	AACGCGAATT	AGGTTATAAA	ACGGAAAAAA	GATATAGTTT	TTTGAAAATG						
2405	2410	2415	2420	2425	2430	2435	2440	2445	2450	2455	2460
AGTTAGCAGG	GATGTTCCCT	ACCGAGGATT	TTTAACCCCC	AATCTCTCAT	AATCGCTAGT						
TCAATCGTCC	CTACAAGGAA	TGGCTCCTAA	AAATTGGGGG	TTAGAGAGTA	TTAGCGATCA						
2465	2470	2475	2480	2485	2490	2495	2500	2505	2510	2515	2520
GTTTAAAAGG	CTAAGAATAG	TGGGGCCCAA	CCGATGTGGT	AGGTGATAAA	GAGGCATCTT						
CAAATTTTCC	GATTCTTATC	ACCCCGGGTT	GGCTACACCA	TCCACTATTT	CTCCGTAGAA						
2525	2530	2535	2540	2545	2550	2555	2560	2565	2570	2575	2580
TTCTAGAGAC	ACATTGGACC	AGATGAGGAT	CCGAAACGGC	AGCCTTTACG	TTTCATCACCT						
AAGATCTCTG	TGTAACCTGG	TCTACTCCTA	GGCTTTGCCG	TCGGAAATGC	AAGTAGTGGA						
2585	2590	2595	2600	2605	2610	2615	2620	2625	2630	2635	2640
GCTAGAACCT	CTCGTAGTCC	ATCACCATT	CTTGCCATTG	GAATTCCTACT	GGAAAAAAT						
CGATCTTGGA	GAGCATCAGG	TAGTGGTAAA	GAACCGTAAC	CTTAAGATGA	CCTTTTTTTA						
2645	2650	2655	2660	2665	2670	2675	2680	2685	2690	2695	2700
ACAAAAAGCA	AAACAAAACC	CTCAGCACTG	TTACAAGAGG	CCATTTAAGT	ATCTTGTGCT						
TGTTTTTCGT	TTTGTTTTGG	GAGTCGTGAC	AATGTTCTCC	GGTAAATTCA	TAGAACACGA						
2705	2710	2715	2720	2725	2730	2735	2740	2745	2750	2755	2760
TCTTCACTTA	CCCATTAGCC	AGGTTCTCAT	TAGGTTTTGC	TTGGGCCTCC	CTGGCACTGA						
AGAAGTGAAT	GGGTAATCGG	TCCAAGAGTA	ATCCAAAACG	AACCCGGAGG	GACCGTGACT						
2765	2770	2775	2780	2785	2790	2795	2800	2805	2810	2815	2820
ACCTTAGGCT	TTGTATGACA	GTGAAGCAGC	ACTGTGAGTG	GTTCAAGCAC	ACTGGAATAT						
TGGAATCCGA	AACATACTGT	CACTTCGTCT	TGACACTCAC	CAAGTTCGTG	TGACCTTATA						
2825	2830	2835	2840	2845	2850	2855	2860	2865	2870	2875	2880
AAAACAGTCA	TGGCCTGAGA	TGCAGGTGAT	GCCATTACAG	AACCAAATCG	TGGCACGTAT						
TTTGTTCAGT	ACCGGACTCT	ACGTCCACTA	CGGTAATGTC	TTGGTTTAGC	ACCGTGCCATA						
2885	2890	2895	2900	2905	2910	2915	2920	2925	2930	2935	2940
TGCTGTGTCT	CCTCTCAGAG	TGACAGTCAT	AAATACTGTC	AAACAATAAA	GGGAGAATGG						
ACGACACAGA	GGAGAGTCTC	ACTGTCACTA	TTTATGACAG	TTTGTATTAT	CCCTCTTACC						
2945	2950	2955	2960	2965	2970	2975	2980	2985	2990	2995	3000
TGCTGTTTAA	AGTCACATCC	CTGTAAATTG	CAGAATTCAA	AAGTGATTAT	CTCTTTGATC						
ACGACAAATT	TCAGTGTAGG	GACATTTAAC	GTCTTAAGTT	TTCACTAATA	GAGAAACTAG						
3005	3010	3015	3020	3025	3030	3035	3040	3045	3050	3055	3060
TACTTGCCCTC	ATTTCCCTAT	CTTCTCCCCC	ACGGTATCCT	AAACTTTAGA	CTTCCCCTG						
ATGAACGGAG	TAAAGGGATA	GAAGAGGGGG	TGCCATAGGA	TTTGAAATCT	GAAGGGTGAC						
3065	3070	3075	3080	3085	3090	3095	3100	3105	3110	3115	3120

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FIG. 8 - CONT'D

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TTCTGAAAGG AGACATTGCT CTATGTCTGC CTTCGACCAC AGCAAGCCAT CATCCTCCAT
AAGACTTTCC TCTGTAACGA GATACAGACG GAAGCTGGTG TCGTTCCGTA GTAGGAGGTA

3125 3130 3135 3140 3145 3150 3155 3160 3165 3170 3175 3180
* * * * *
TGCTCCCGGG GACTCAAGAG GAATCTGTTT CTCTGCTGTC AACTTCCCAT CTGGCTCAGC
ACGAGGGCCC CTGAGTTCTC CTTAGACAAA GAGACGACAG TTGAAGGGTA GACCGAGTCG

3185 3190 3195 3200 3205 3210 3215 3220 3225 3230 3235 3240
* * * * *
ATAGGGTCAC TTTGCCATTA TGCAAATGGA GATAAAAGCA ATTCTGGCTG TCCAGGAGCT
TATCCCAGTG AAACGGTAAT ACGTTTACCT CTATTTTCGT TAAGACCGAC AGGTCCTCGA

3245 3250 3255 3260 3265 3270 3275 3280 3285 3290 3295 3300
* * * * *
AATCTGACCG TTCTATTGTG TGGATGACCA CATAAGAAGG CAATTTTAGT GTATTAATCA
TTAGACTGGC AAGATAACAC ACCTACTGGT GTATTCCTCC GTTAAAATCA CATAATTAGT

3305 3310 3315 3320 3325 3330 3335 3340 3345 3350 3355 3360
* * * * *
TAGATTATTA TAAACTATAA ACTTAAGGCC AAGGAGTTTA TTACAATGTA TCTTTATTAA
ATCTAATAAT ATTTGATATT TGAATCCCG TTCTCAAAT AATGTTACAT AGAAATAATT

3365 3370 3375 3380 3385 3390 3395 3400 3405 3410 3415 3420
* * * * *
AACAAAAGGG TGTATAGTGT TCACAAACTG TGAAAATAGT GTAAGAACTG TACATTGTGA
TTGTTTTCCC ACATATCACA AGTGTTTGAC ACTTTTATCA CATTCTTGAC ATGTAACACT

3425 3430 3435 3440 3445 3450 3455 3460 3465 3470 3475 3480
* * * * *
GCTCTGGTTA TTTTCTCTTT GTACCATAGA AAAATGTATA AAAATTATCA AAAAGCTAAT
CGAGACCAAT AAAAAGAGAA CATGGTATCT TTTTACATAT TTTTAATAGT TTTTCGATTA

3485 3490 3495 3500 3505 3510 3515 3520 3525 3530 3535 3540
* * * * *
GTGCAGGGAT ATTGCCTTAT TTGTCGTAA AAAATGGAGC TCAGTAACAT AACTGCTTCT
CACGTCCCTA TAACGGAATA AACAGACATT TTTTACCTCG AGTCATTGTA TTGACGAAGA

3545 3550 3555 3560 3565 3570 3575
* * * * *
TGGAGCTTTG GAATATTTTA TCCTGTATTC TTGTTT (SEQ ID NO:9)
ACCTCGAAAC CTTATAAAAT AGGACATAAG AACAAA

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/01078

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 14/435, 16/00; C07H 21/04; C12Q 1/68; G01N 33/53

US CL : 536/23.2; 530/387.1; 530/350; 435/6; 436/7.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.2; 530/387.1; 530/350; 435/6; 436/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MEDLINE, WPI, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A, 94/24159 (NATIONAL JEWISH CENTER FOR IMMUNOLOGY AND RESPIRATORY MEDICINE) 27 October 1994, see pages 8, 16, 18, 21, 22, 28, 30, 38, 59, 60.	1, 22-37
Y	Journal Of Biological Chemistry, Volume 267, No. 36, issued 25 December 1992, Seger et al, "Human T-cell Mitogen-Activated Protein Kinases Are Related To Yeast Signal Transduction Kinases", pages 25628-25631, especially page 25630.	29
Y	Molecular And Cellular Biology, Volume 13, No. 8, issued August 1993, Wu et al, "Identification and Characterization of a New Mammalian Mitogen-Activated Protein Kinase Kinase, MKK2", pages 4539-4548, especially pages 4542 and 4543.	24, 28-32



Further documents are listed in the continuation of Box C.



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"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

08 MAY 1996

Date of mailing of the international search report

24 MAY 1996

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/01078

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 14/435, 16/00; C07H 21/04; C12Q 1/68; G01N 33/53
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.2; 530/387.1; 530/350; 435/6; 436/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MEDLINE, WPI, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	Journal Of Biological Chemistry, Volume 267, No. 36, issued 25 December 1992, Seger et al, "Human T-cell Mitogen-Activated Protein Kinases Are Related To Yeast Signal Transduction Kinases", pages 25628-25631, especially page 25630.	29
Y	Molecular And Cellular Biology, Volume 13, No. 8, issued August 1993, Wu et al, "Identification and Characterization of a New Mammalian Mitogen-Activated Protein Kinase Kinase, MKK2", pages 4539-4548, especially pages 4542 and 4543.	24, 28-32

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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
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Date of the actual completion of the international search

08 MAY 1996

Date of mailing of the international search report

24 MAY 1996

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